No. 4, 1912.

MINISTRY OF INTERIOR.

PUBLIC HEALTH DEPARTMENT.

DESCRIPTION

OF THE

SERUM INSTITUTE (Abbassîa)

AND OF THE

PREPARATION OF CATTLE PLAGUE SERUM,

BY

CHARLES TODD, M.D.

Bacteriologist, Public Health Department, Egyptian Government.

(From the Hygienic Institute, Public Health Department, Cairo).

CAIRO:
Government Press.

To be obtained, either directly or through any Bookseller,
from the Publications Office, Government Press, Bulaq; from the Sale-Room, Geological Museum,
Ministry of Public Works Gardens; or from the Survey Department, Giza (Mudiria).

1912

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INTRODUCTION.

The conditions under which the preparation of Cattle Plague serum have to be undertaken differ, as a rule, materially from those under which the sera ordinarily in use are manufactured.

A sudden outbreak of the disease in a country which for many years has been free from Cattle Plague creates an urgent demand for serum, which must be provided as soon as possible and in large quantities. A staff must be got together and trained, temporary buildings erected, and the process of manufacture instituted without delay.

The literature dealing with Cattle Plague serum does not contain much information on the actual practical methods, and such information is not easy to obtain. Consequently, a technique has to be elaborated and practical difficulties met as they arise. In the light of the experience subsequently gained many of these difficulties which were met with on beginning the Institute at Abbassîa might have been avoided.

For this reason, in the following account of the Serum Institute and of the method of preparation of the serum, many small details are given which may perhaps appear somewhat unnecessary.

NOTE.—This description of the Serum Institute was written when the Institute was closed at the end of 1905.

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THE SERUM INSTITUTE (Abbassîa).

DESCRIPTION.

The Serum Institute was situated on the summit of one of the group of small hills forming the Gebel Ahmar in the Arabian Desert and separated by about a mile from Abbassîa, then the most eastern suburb of Cairo; it thus had the advantage of being well isolated and at the same time of being within about four miles of the centre of Cairo, a very important point in view of the large quantities of supplies (forage, etc.) required, and the question of transport and of water supply.

Originally intended for the preparation of serum against human plague on a comparatively small scale, it was gradually enlarged as the demand for Cattle Plague serum arose, and finally occupied a considerable area and was capable of accommodating more than five hundred animals.

The annexed plan shows the general arrangements; these are perhaps not quite what would have been chosen had the Institute been completely erected at once and not gradually evolved from a small nucleus, but on the whole they were found to work remarkably well.

The stabling for the serum animals, together with the buildings more especially concerned with the treatment of these animals (*e.g.*, reinforcing and bleeding rooms, etc.), with offices and stores were included in a large compound surrounded by a high fence. Outside this at some little distance and well separated from one another were the Quarantine and Isolation stables, stabling for infected Cyprus cattle, slaughter-house, accommodation for staff, etc.

STABLING.

The stabling in the compound was arranged as far as possible in long rows running approximately north and south. As the prevailing wind in Cairo all the year round is from the north this arrangement ensured a free current of air between the different lines. Each row consisted of four separate stables constructed of wooden battens covered with matting which was rolled up at the sides during the summer to allow of free access of air, and rolled down so as to completely enclose the stables in winter. This was found very necessary as the evening temperature in winter sometimes fell to freezing point, and cattle undergoing the treatment do not thrive unless kept under reasonably comfortable conditions.

The stables were 5 metres high, 5 metres broad, and a length of 2 metres was allowed for each animal. A wooden manger ran throughout divided by partitions into separate feeding places, and the cattle were fitted with head-stalls and by these attached by a short length of rope to rings in the mangers. At first the Egyptian method of tying up the animals by a rope round the base of the horns was tried, but was found very unsatisfactory for several reasons especially from the fact that the area at the root of the horns covered by the rope formed an excellent shelter for ticks. The cattle were branded on the rump with their individual numbers and were all ringed: the ring proving very convenient in handling restive animals and also serving the purpose of identification in case of the branding becoming indistinct, as each ring was stamped with the animal's number. Down the centre of the spaces between the lines of stabling were fixed picket ropes to which the animals were tethered during the daytime when the stables were being cleaned.

The stables being built on dry stony ground no special flooring was necessary beyond the removal of any large stones and the laying down of sand of which there was an abundance in the immediate vicinity of the Institute. As rain is rare and the climate excessively dry this arrangement worked excellently and no bedding other than clean dry sand was required. All manure was removed as dropped during the day, and first thing in the morning the cattle were taken out of the stables and tied up to the picket ropes whilst the stables were thoroughly cleaned.

In this way the stables were found to keep quite sweet and dry. Outside the compound were two lines of stabling, one of which was used as a quarantine stable and the other as Sick Lines.

Further south was another block of stabling for infected Cyprus animals, and again at some little distance were two small stables used for the isolation of any suspected cases of a contagious nature other than Cattle Plague.

About half a mile from the Institute were the stables for clean Cyprus cattle; these stables were strictly isolated and had a separate staff of stable-men. The greatest care was taken to prevent any intercourse between the stable-men of the Institute and those of these stables. Separate arrangements were made for the forage supply to these stables, food of men, etc.

The total stabling accommodation was as follows:—

Compound	416
Sick Lines	30
Quarantine	30
Infected Cyprus Animals	40
Clean " "	40
Isolation	12
Total	568

Inside the compound were also the Bleeding Rooms, Reinforcing Rooms (the arrangements of which are referred to later), Pharmacy for drugs, antiseptics, and instruments, room for washing glass and other apparatus, room for ice safes, glass store, general store, store for paraffin and spirit, temperature-chart room and offices for Inspectors and clerk, carpenters' shop and stabling for donkeys and mules.

As all the above buildings were of wood great precautions had to be taken against fire; smoking in the compound was of course strictly prohibited and no fires could be used. Petroleum and alcohol had to be employed for all the sterilising, and to minimise the risk a special sterilising room of brick with concrete floor and asbestos roof was erected. All the sterilising was done in this room. For the larger articles (glass, etc.) two large simple steam sterilisers were used: these were heated by Primus lamps burning paraffin and worked very well; for smaller articles (instruments, etc.) ordinary Schimmelbusch sterilisers heated by spirit were employed.

WATER SUPPLY, DRAINAGE, ETC.

The town supply* of unfiltered Nile water was brought to the foot of the hill on which the Institute was situated and from there was pumped up by a hot-air engine into large galvanised iron tanks which were erected on stageing under matting shelters just outside the compound. From these tanks the water was supplied to a long concrete drinking trough. All water used for washing, diluting antiseptics, drinking, etc., was filtered through a large Berkefeld filter fitted with a hand pump and hose.

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* The Cairo water supply includes two separate distributions: (1) from deep wells for domestic purposes, and (2) unfiltered Nile water for general purposes other than domestic.

The only drains in the compound were two short drains leading, from the Bleeding and Reinforcing Rooms respectively, into concrete sumps immediately outside these buildings. These sumps were emptied and cleaned out every day as soon as the morning's work was over. The whole area of the compound was regularly watered by a water cart and swept with a machine brush, this being in addition to the ordinary hand sweeping.

FORAGE.

A large forage store (48 metres long by 6 metres broad) was placed inside the compound at some distance from the stabling, in view of the possibility of fire, and the forage was brought up by carts from Cairo.

The store contained separate rooms for the different kinds of forage and was large enough to contain an adequate reserve.

It was arranged that the reserve of dry forage should never fall below a minimum of three days' complete supply.

Immediately in front of the store was a large weighing machine on which all forage was weighed on arrival and again on issue to the various stables.

DISPOSAL OF MANURE.

All manure, stable refuse, etc., was dumped in a valley in the desert about a quarter of a mile to the south of the compound: it was brought there in tip-trucks drawn by a mule or donkey on a light railway which ran between the various stables and also communicated with the Bleeding Rooms, the Abattoir, etc., and so could also be employed for the removal of carcases from the Bleeding Rooms to the Abattoir.

Under the influence of the sun and excessive dryness the manure became dry very quickly, and being to leeward of the Institute gave rise to no nuisance except in so far as it served as the breeding place of innumerable flies.

STAFF.

In a country like Egypt the assembling of a suitable staff for the rather specialised work of serum production was a matter of some little difficulty, but by judicious selection and elimination this difficulty was overcome.

Fortunately, almost every Egyptian fellah is accustomed to handling cattle from his earliest childhood, so that ordinary stable-men were not difficult to obtain, and after a little time in the Institute they adapted themselves very well to the conditions of the work. They required, however, constant supervision, and this was carried out by two English Inspectors who were responsible for the stabling and care of the animals, superintended the reception and issue of forage, watering of animals, etc., and in fact were responsible for the general condition of the animals and non-scientific work.

The health of the animals and the processes of reinforcing, bleeding, etc., were under the charge of two English Veterinary Surgeons who had under them a staff of seven untrained but educated assistants of various nationalities, Egyptian, French, Greek, etc. These were all men who had been through a course at the Agricultural College; they undertook the reinforcing, bleeding, etc., and superintended the temperature taking and kept the temperature charts. After a little experience they became very expert at such operations as bleeding and reinforcing.

The numbers of the staff were as follows:—

Director	1
Veterinary Inspectors	2
Inspectors	2
Clerk	1
Temperature-chart Clerk	1
Assistants	7
Natives in Sterilising Rooms	5
Natives in Bleeding Rooms	12
Natives in Reinforcing Rooms	6
Sweeper	1
Shawîsh	1
Carpenter	1
Engine Drivers	2
Stable-men	52
Total	100

The number of stable-men was altered from time to time according to the number of animals at the time in the Institute.

The English Inspectors and the Assistants were housed in tents outside the compound, the stable-men sleeping in the stables with their animals. The gates of the compound were locked at night and a service of watchmen arranged to patrol the outside of the compound.

The food of the Assistants and stable-men was supplied from the Hospital at Abbassîa and was sent up ready cooked. This did away with the necessity of fires and was a great convenience.

A small shed with water supply was provided for the ablutions of the stable-men: this was necessary as before its erection they had a habit of using the drinking troughs for the purpose.

The general work of the staff was found to be much simplified by allotting to each man definite duties to be performed according to a fixed time-table. Foremen were appointed over the various gangs and a strict discipline maintained.

TYPE OF CATTLE USED.

The animals used for the production of serum were carefully chosen for the purpose by the Veterinary Department.

Lower Egypt animals were preferred as being more or less immune to many parasitic diseases of the country, *e.g.*, Texas Fever, Egyptian Fever, etc. Only large healthy animals were chosen and young animals were avoided as it was found that these did not stand the strain of immunisation as well as older cattle. The age found best was from five years onwards, animals of four years or less were not found satisfactory.

On arrival at the Institute they were carefully searched for ticks, branded and ringed, and placed in the Quarantine Stables for a fortnight. During this time they were of course regularly groomed and searched for ticks.

At the end of this period if all went well they were transferred to the compound.

STABLING, FEEDING, AND CARE OF ANIMALS.

After temperature taking at 7 a.m. the cattle were removed from the stables and picketed to ropes between the lines of stabling and were here groomed and any ticks found removed. Whilst they remained outside, the stables were cleaned out and allowed to dry and air, the refuse being removed by means of tip-trucks on a light railway and taken outside the compound.

After remaining in the open air for a certain time, depending on the season of the year, the animals were led back into the stables for their mid-day feed. At intervals during the day they were inspected, and any found sick removed to the Sick Lines for treatment.

During the winter they were watered in the morning and evening only, but in summer it was found that they required water three times a day.

The average quantity of water drunk by an Egyptian bull in hot weather was found to be approximately 50 litres per day. This was determined by allowing 50 bulls to drink at a measured trough three times during the day and measuring the fall in the water level. They were given full liberty to take as much as they liked. The day (June 15, 1905) was very hot.

The average amount drunk per head was :—

Morning (6 a.m.)	10	litres.
Noon	20	„
Evening (4 p.m.)	19	„
Total...	49	„

The forage allowance of the cattle was liberal and consisted of "tibn" (the chopped wheat straw of the country), with green "bersîm" (*Trifolium Alexandrinum*) during the spring and summer months, and "drees" (dried "bersîm") during autumn and winter. Beans were given regularly with a certain amount of cotton-cake and a block of crude salt was placed in each animal's manger.

The forage scale was drawn up by the Veterinary Department and was at first made intentionally too liberal, it was then reduced to what it was found the animals could eat. The final ration is appended.

For the ordinary serum animals one stable-man was allotted to each ten cattle, but in the case of animals requiring more attention, as in the case of new animals and those in the Sick Lines, the proportion was one to eight.

Very thorough grooming was insisted on and ticks were carefully searched for, any found being put into small bottles containing alcohol or paraffin.

For the satisfactory production of serum over any length of time it is most important to maintain the animals in good condition by paying the utmost attention to their feeding, housing, and general well-being; too much stress can hardly be laid on this point. Any animal showing signs of losing condition must be given a period of rest until he picks up again.

Under such conditions it is surprising how well the animals stand the treatment: they look sleek well and confortable in spite of the fact that they are being bled to the extent of four or five litres every fortnight.

The temperatures of all the animals in the Institute were taken twice a day regularly and charted in special books.

This involved a large amount of work but was found a very great assistance and in fact quite essential.

The method of taking the temperature was as follows:—

About 50 cattle were allotted to each Assistant who went round these animals with a small gang of stable-men, each provided with a half-minute thermometer. At a signal from the Assistant the thermometers were inserted, and at another signal, at the expiration of a minute, they were removed and brought to the Assistant who entered the temperatures in a note book to be subsequently charted. In this way the process could be got through very rapidly and the temperatures were all charted and ready for inspection by 8-30 a.m., before the bleeding for serum and the reinforcing began.

The temperatures were checked from time to time by the Veterinary Surgeons, to be certain that they were being correctly taken.

PRODUCTION OF SERUM.

General Sketch of Process.

The method employed for the preparation of the serum was that worked out by Koch and elaborated by Kolle and Turner, the technique being modified to suit the conditions of the country.

In brief the method is as follows :—

Clean susceptible cattle are inoculated with a small quantity of virulent Cattle Plague blood, and after a short incubation period they contract a severe attack of the disease. When this is at its height they are bled to death, and the virulent blood so obtained, after defibrination or the addition of Citrate of Potash to prevent clotting, is used for the treatment of the animals designed for the production of serum. For this purpose healthy cattle are taken and given simultaneous inoculations of 1 cc. of virulent blood on one side of the body and a suitable dose of serum on the other side. These injections are followed by a modified attack of the disease from which, if the dose of serum has been properly chosen, the animal recovers. After complete recovery and when the temperature is normal a massive dose of four litres of the virulent blood is injected subcutaneously or intramuscularly. This again is followed by a febrile reaction, after the termination of which another dose of four litres of virulent blood is given.

A fortnight after the last injection the animal is bled—four litres being taken from the jugular vein. Three such bleedings, at intervals of a fortnight, are taken, and then another dose of four litres of virulent blood given and the process repeated. The blood thus taken is allowed to clot and the serum decanted, centrifugalised and bottled.

The various steps in the process may now be considered more in detail :—

Production of Virulent Blood.

It is obviously desirable that the virulent blood employed, while containing the agent of Cattle Plague in as great an amount as possible, should be free from parasites causing other diseases. When the preparation of serum was first commenced in Egypt native cattle were employed for the production of virulent blood and serious dificulties were encountered from Texas and Egyptian Fevers. The majority of Egyptian cattle have at some time or other gone through an attack of Egyptian Fever and many of them have also had Texas Fever and so possess a certain immunity against these diseases. The effect, however, of an acute attack of Cattle Plague is very often to break down this immunity, with the result that there is an enormous multiplication of the parasites and the blood is quite unfit for the purpose of injection into other animals.

This difficulty was overcome by the use of cattle from Cyprus, this island being at the time free from Cattle Plague. The Cyprus cattle were found to be quite free from Texas Fever and almost so from Egyptian Fever, and also had the advantage of being very highly susceptible to Cattle Plague—of 1,098 animals inoculated with virulent blood only one being found immune, this was a very old cow, whose previous history was of course unknown.

The Cyprus cattle were imported weekly, *via* Alexandria, and were brought up to Cairo in disinfected railway trucks and kept strictly isolated in a stable about half a mile from the Institute. By taking great care to prevent, as far as possible, all communication between this stable and the Institute, practically no trouble was experienced from these animals catching the disease. On two occasions Cattle Plague broke out in the Cyprus stables but could be traced to a probable infection either on board ship, in Alexandria, or during the train journey.

The Cyprus animals were brought up to the Institute daily as required and were there at once inoculated with a small dose of virulent blood.

COURSE OF DISEASE IN CYPRUS ANIMALS.

The following description of the symptoms of the experimental disease in Cyprus animals is Furnished by Mr. Rabagliati, one of the Veterinary Inspectors at the Institute.

Symptoms Observed in Cyprus Cattle Inoculated with Virulent Cattle-Plague Blood.

"The animals were inoculated with varying quantities of virulent blood, anything from 1 cc. "to 10 cc. being used.

"This was usually given subcutaneously over the ribs.

"No abnormal symptoms were noticed for the first two days at least after inoculation.

"The first abnormal symptom was a rise of temperature, most commonly on the evening of the "third day after inoculation, but latterly when apparently the strain of blood became more virulent, "the temperature rose on the morning of the third day, or sometimes on the evening of the second.

"In a few rare cases the temperature remained normal until the evening of the fourth day "after inoculation.

"The rise of temperature was generally abrupt, rising to at least 40° C., more commonly to "40·5° C. or 41° C. With this rise there were rarely any clinical symptoms noticeable, the animal "continuing to eat and drink as usual, and all the other bodily functions remaining normal.

"On the evening of the fourth day the animal began to look dull, the coat became dry and "staring, sometimes there was some constipation, the fæces being dry and hard or even altogether "absent. On examining the mucous membranes, these were found to be red and congested, "especially the conjunctivæ which were of a bright scarlet colour.

"The respirations were slightly increased and the pulse full with 60–70 beats per minute. "The muffle was dry and hot and sometimes there was a slight nasal discharge.

"Up to this period the appetite was usually well maintained.

"On the fifth day the above symptoms were increased, temperature 41°–42° C., but in some "cases remaining between 40° and 41° C. Respirations rapid, 60–80 per minute, pulse 80–100, the "animal stood with head and neck extended, heaving flanks, tucked up belly, and had a very "haggard expression. The eye looked dull and sunken and in some cases there was a discharge "of tears which matted the hair of the cheeks, though in a great many cases the tears were "completely absent. Conjunctivæ still bright red, Schneiderian mucous membranes red with a "usually sticky and dirty discharge from the nose which collected about the muffle, giving it a "dirty appearance, sometimes causing the epithelium to peel off in patches, leaving raw sore eroded "areas. By the evening of the fifth day there was usually complete loss of appetite; and in many "cases diarrhœa had set in, though this was the exception rather than the rule. When the animal "had been in very bad condition to commence with, he often lay down a good deal and rose only "when roused and even then with difficulty.

"By the sixth day the animals (especially latterly) were bled to death in the morning, this "being done from the carotid artery. The quantity of blood obtained varied from 8 to 12 litres, "in rare cases being as much as 16 litres.

"When not killed the following differences in the symptoms were apparent from those of the "fifth day:—

"Emaciation was more marked, there was a more draggled appearance, the hind extremities "being dirty and covered with the diarrhœic discharge which often contained blood, especially "when the diarrhœa had set in early. There was great straining and often eversion of the rectum, "which was quite relaxed, the animal passing liquid and bloodstained fæces involuntarily. Loud "borborigmi were quite common along with the passage of flatus. It was often found difficult "to register the rectal temperature owing to the non-closure of the sphincter ani.

"The respirations were often very rapid, sometimes over 100 per minute, and the pulse was "very rapid and weak.

"In many cases, however, the diarrhœa only commenced on the sixth day, and in these cases "all other symptoms were less aggravated.

"When the animals were not bled to death until the seventh day they were often only brought "to the Bleeding Room with much difficulty, being too weak to walk without aid in many cases, "and a very much smaller quantity of blood was obtained from them: it rarely exceeded 10 litres "and more usually only 8 litres were obtained, and in some cases only 4.

"Most of the animals used were in very poor condition to begin with, and the worse their "condition the earlier the Cattle Plague symptoms exhibited themselves, especially the weakness. "Occasionally animals were seen on the sixth day unable to rise and with very severe diarrhœa, "whereas in other cases, apart from the rise of temperature and some loss of appetite, it would have "been difficult to say that they were ill. It was always noticed that a great number of the cattle "had no discharge from the eyes, and many had a complete absence of, or only a very slight nasal "discharge.

"Ulceration of the mucous membrane of the lips and mouth was extremely rare, a very "limited number of cases being seen with it, and the cutaneous eruption, apart from a dry and "unhealthy condition of the skin, was only once or twice observed. The fact that the animals "were in very poor and rough condition to begin with made the conditions of the skin such as "the hard and dry nature of the coat less significant.

"The symptoms seemed to advance rapidly between the evenings of the fifth and sixth days, "a greater difference in condition being noticed in this period than in any other of equal duration.

"*Autopsy.*—The chief lesions looked for before using the blood for serum animals were "those of the abomasum and of the small intestines, the character of the bile and the condition of "the spleen.

"*Carcase.*—Usually found to be much emaciated and the flesh dark coloured, soft and soapy "to the touch with a great absence of internal fat.

"*Abomasum.*—The condition of the mucous lining varied very much. The most general "condition found on the sixth day was a slight patchy congestion around the pylorus. This was "not often of a very dark colour, but varied from very slight pink to in rare cases very characteristic "Cattle Plague ulcers. The rest of the mucous membrane was pale, often rather slimy and slightly "yellowish.

"In a great many cases examined small rounded or irregularly elongated ulcers occurred, "either on the sides of the leaves or round the pylorus, or both. They were deeply penetrating "to the submucosa, black in colour, and varied in size, the round ones being from one to two "millimetres and the elongated up to one centimetre in diameter. These, however, were not "considered as having any connection with Cattle Plague.

"In other cases the whole of the mucous membranes of the stomach was congested and of a "deep red colour.

"In those cases killed on the seventh day the lesions were often more advanced.

"*Small Intestines.*—In some cases showed congestion of all the coats with characteristic zebra "striping of the mucous membrane. In many cases they showed very little change from the "normal though the mucous membrane was sometimes covered with a slimy yellowish mucoid "exudate, especially in those animals killed on the sixth day.

"*Large Intestines.*—Showed very slight changes, though at times there was striping of the "mucous membrane, the stripes running longitudinally and not transversely as in the small "intestines.

"*Bile.*—In early cases and when the other lesions were slight this was often of a deep green "colour and usually clear. When the lesions were more advanced, as in those animals killed on "the seventh day, it was often reddish brown or yellowish in colour, sometimes thick and turbid. "The bright green "Cattle Plague bile" was by no means the rule.

"*Spleen.*—This was usually normal, though occasionally enlarged, in which case the blood "was always rejected.

"Ulcers on the septum nasi or about the nose were very rare.

"*Note.*—Very frequently, cases showing very slight symptoms while alive had the most "pronounced lesions on autopsy and *vice-versa*, especially those killed on the sixth day.

"If left after the seventh day, the lesions were usually more advanced and more characteristic.

"Many autopsies were made from which it would have been quite impossible to diagnose "Cattle Plague had no history been known and the temperature not risen in the characteristic "fashion.

"Blood for continuing the strain in Cyprus animals as a rule was taken from the cases "showing most pronounced lesions, but when inoculations were made from blood taken from cases "with practically no lesions, so long as the temperature reaction had been good, the virulence of "this blood seemed equal to that of the blood from the worst cases.

"In those cases which were not bled to death, but allowed to die of the disease, death took "place in from eight to twelve days after inoculation, perhaps death on the ninth day was the "most common."

TYPICAL COURSE OF TEMPERATURE.

On comparing the temperature charts of a large number of cases of the experimentally produced disease one is struck by the remarkable similarity of the temperature curves. The most characteristic point being the almost constant incubation period before the initial rise of temperature. These facts are well shown in the annexed charts.

To eliminate small accidental variations the temperature charts of groups of four consecutive animals (without any selection) were taken. Beginning with the first day, the morning temperatures of each of the four animals were added together and divided by four, thus giving the average temperature of the four animals for that morning. This was repeated for the morning and evening observations of six days and the average temperature charted on one chart which was the average temperature chart for four animals.

Chart 1 (*a*, *b*, *c*, *d*) shows a group of four of these charts each representing the average of a group of four animals. The similarity is very striking.

Chart 2 is a chart similarly obtained by averaging the temperature charts of a series of 20 consecutive Cyprus animals and may be taken as the typical chart of the experimentally produced disease in Cyprus animals.

From this it will be seen that the first rise of temperature takes place with great regularity on the evening of the third day, *i.e.*, 72 hours after the inoculation.

To further establish this a series of 637 charts of experimentally infected Cyprus animals was examined and a note made of the exact period at which the first rise of temperature occurred. The results are as follows:—

Rise of Temperature.	No. of Animals.	Percentage (approx.).
On 1st day	0	0
On 2nd day	97	15
On 3rd day	437	69
On 4th day	94	14·5
On 5th day	9	1·5
On 6th day	0	0

The injections of virulent blood were unfortunately not made at the same hour every day during the period under observation but varied from 9 a.m. to 6 p.m., and but for this fact the results would probably have been even more striking. The few cases in which the rise did not occur until the fifth day were probably largely accounted for by some accidental circumstance, *e.g.*, the thermometer not being left sufficiently long in the rectum; moreover, in winter the Cyprus stables were very much exposed to the cold south wind, and as the animals were usually in very poor condition they not infrequently showed a subnormal temperature.

This typical temperature curve is most valuable in the diagnosis of the disease, and in cases where an experimental inoculation is made for diagnostic purposes it is the temperature chart as a rule which gives the most useful evidence. That this is also the case in other parts of the world is shown by Yersin's statement *à propos* of the epizootic in Indo-China; he says:—

"Les seuls symptomes constants, que nous avons presque toujours observés chez les animaux "infectés par nous, ont été la durée de l'incubation, la période d'hyperthermie sans rémission, et "la diarrhée."

BLEEDING TO DEATH FOR VIRULENT BLOOD.

The infected Cyprus animals were bled to death as a rule six days after the inoculation of virulent blood.

The animal to be bled was walked into the Bleeding Room, thrown on a large straw mattress covered with a waterproof sheet and the four legs tied together.

An incision was then made through the skin and subcutaneous tissue of the lower third of the neck over and parallel to the course of the carotid artery. With the fingers in the wound the artery was felt, drawn out of the wound by means of a small blunt-hook and cleared from its sheath.

Two ligatures were placed round the artery at a short distance from one another, the distal ligature being at once tied and the proximal one afterwards used to tie in the canula. A longitudinal incision was made in the wall of the artery and through this incision a blunt canula was inserted and at once tied in place. The other end of the canula was connected with about a metre and a half of rubber tubing by means of which the blood was run into large ten-litre glass bottles containing a certain amount of Citrate of Soda solution. The amount of Citrate of Soda was such that it represented about 0·5% of the blood. This amount is somewhat in excess of that actually required but was not found to be in any way harmful.

During the process of bleeding the bottle was gently rocked from side to side so as to ensure thorough mixing of the blood and Citrate solution. The large bottles containing the necessary amount of Citrate solution were plugged with cotton-wool and sterilised in the steam steriliser before use.

By this method there was no difficulty in obtaining the blood perfectly sterile. Previously to employing Citrate the blood was run into whipping machines holding about 10 litres (as employed by confectioners for mixing batter) and so defibrinated, but this necessitated subsequent filtration through muslin to get rid of fibrin and consequent risk of contamination ; moreover, the whipping machines were more or less complicated and troublesome to clean.

The amount of blood varied very much with the size of the animal, period of the disease, etc., but as a rule averaged about 13 litres, the weight of the animals averaging about 400 kilos, though this was exceedingly variable.

Bleeding for virulent blood took place between 7 a.m. and 8 a.m. and the blood so obtained was placed in the ice safe until the autopsies of the animals had been made; if these were satisfactory it was then used for reinforcing.

A light railway ran from the Bleeding Rooms to the Abattoir so that the carcases could be rapidly removed for examination.

All hides were soaked for 24 hours in a large tank of 2% soap-cresol solution before they were allowed to leave the Institute.

At different times several strains of virulent blood from different parts of the country were experimented with to see if any difference in virulence could be observed, but at any rate for the Cyprus animal, this did not appear to be the case, and for the routine work one strain was adhered to. After passage through Cyprus cattle for about eighteen months no noticeable change was obvious in the course of the disease, unless as mentioned above there was a slight shortening of the incubation period before the first rise of temperature. If this shortening of the incubation period did take place, however, it could only have been one of a few hours.

In making use of a new strain of Cattle Plague by inoculating Cyprus animals with blood from Egyptian animals suffering from the natural disease great trouble was experienced at first from Texas Fever, etc., but this trouble was subsequently avoided by infecting the Cyprus animals in the first case not by subcutaneous injection but by smearing the nose with nasal mucus or other virulent matter. This method is quite certain but the incubation period is somewhat longer and the temperature does not rise until about four days after infection—usually the evening of the fourth or the morning of the fifth day.

In order to secure a regular supply of virulent blood, two, three or four Cyprus cattle were inoculated every day according to the amount of blood required. For this purpose fresh virulent blood was used, but experiments showed that the blood retained its virulence for at least seven days if kept in the ice-safe. A longer period was not tried.

The sixth day was ultimately chosen as the best day for bleeding to death for several reasons. If left until the seventh day the disease was often so far advanced that the amount of blood obtained was very small and there was always the risk of a secondary terminal infection.

On the sixth day the blood was highly virulent—a series of tests made by diluting the virulent blood showed that 1/500 cc. of blood taken on this day and injected subcutaneously into a Cyprus animal gave rise to the typical disease and death. Smaller quantities were unfortunately not tried. These tests were begun with the idea of determining the relative virulence (quantitatively) of the blood on each day of the disease, but at the time they were done the demand for serum was so urgent that it was found impossible to risk upsetting the routine of the work, which would have been the case if several of the animals had failed to react; consequently, the tests had to be stopped before reaching very small quantities. The dose of 1/500 cc., however, agrees with what was found by Koch and Nicolle.

The former succeeding with 1/500 cc. and the latter, in one animal, with 1/000 cc.

Yersin (Annales de l'Institut Pasteur, 1904, p. 427) observed in Indo-China that in several cases the animals which received small doses contracted a more severe attack than those receiving arger quantities. No evidence of this was seen in the animals inoculated at Abbassîa.

SIMULTANEOUS INOCULATION.

METHOD.

At the time during which the Institute was working Cattle Plague was widely spread throughout the country and it was sometimes found that cattle sent up to Abbassîa to be used for the preparation of Serum were incubating the disease on arrival. For this reason simultaneous inoculation was always done at once and the animals then placed in the Quarantine Stables until it was ascertained that they were not suffering from any contagious disease other than Cattle Plague.

The simultaneous inoculations were made as recommended by Kolle and Turner, 1 cc. of blood being given subcutaneously over the ribs on one side and the Serum in the same situation on the opposite side. The dose of Serum necessary is referred to later in discussing the question of testing the Serum : the dose, of course, varies with the strength of the particular Serum and with the weight of the animal, but in practice 100 cc. was given independently of the weight; this is more than is actually necessary but was found to give good results and the resulting reaction was not too violent.

In cases where the animal arrived at the Institute with a high temperature and was obviously suffering from the disease, a dose of 200 cc. was given.

The results of this simultaneous injection were very different in different animals, some having a very sharp reaction with high temperature, loss of appetite, etc., whilst others showed much milder symptoms and some no trace of reaction. This was probably due to the fact that, as the disease had been for some time in the country, some of the animals had previously had the disease and it is also likely that many of them had received prophylactic injections of Serum at some previous date.

In those animals which possessed some degree of immunity and which had mild reactions the period of incubation before the initial rise of temperature seemed to be prolonged.

Typical temperature charts of two animals which received simultaneous inoculations are appended (Charts 5 and 6, Plate II).

As a rule after a period of fever of varying duration the temperature gradually fell to normal and remained there, but in quite a number of animals a somewhat characteristic "secondary rise" was observed. This was more common in cases where the primary fever was high and the reaction severe and is possibly to be attributed to intestinal lesions.

MORTALITY AND CAUSES OF DEATH.

Of 282 animals receiving simultaneous inoculations 11 died, a mortality of 3·9%, the causes of death being :—

Texas Fever	9
Foot and Mouth Disease...	1
Cattle Plague	1
Total...	11

This somewhat high mortality was almost entirely due to Texas Fever which is very prevalent in Lower Egypt.

It is interesting to note that of the animals which died of Texas Fever, four were in one batch of cattle all from the same locality and three in another batch.

Possibly these animals came from badly infected districts.

In injecting animals which have had Texas Fever there is always a risk that the reaction resulting from the simultaneous inoculations may break down the immunity to the piroplasma and allow the latter to multiply, and for this reason, as so many of the Egyptian cattle have the piroplasma, it was found better to give rather a large dose of Serum in order that the reaction should not be too severe.

Serum prepared at the Institute at Abbassia was used on a considerable scale in the Sudan for the simultaneous inoculation of Sudanese cattle with a mortality of about 2% (Head, "Journal of Comparative Pathology," 1906, Vol. 19, part 1).

REINFORCING.

As soon as the reaction following the simultaneous inoculation was over and the animals had regained their condition they were reinforced with a dose of four litres of virulent blood. In cases where the simultaneous inoculation had not been followed by any reaction, they were reinforced at once, and it is interesting that in no case did any of the animals die of the disease, showing that in the complete absence of any reaction there was a very high degree of immunity.

Every evening a list was made of the animals which were ready for reinforcing on the following day, and early next morning the temperature charts of these were inspected and any showing irregular or high temperatures struck out. The revised list was sent out to the stables and the animals inspected by one of the veterinary surgeons who again struck off any which he considered unfit for reinforcement, either from want of condition, lameness, or other cause.

Reinforcing Rooms.

The method of reinforcing and the arrangement is seen in the photograph of the Reinforcing Rooms. These rooms were of wood with concrete floor and had in the centre of each room wooden stocks for holding the animals, their necks being fixed without pressure by a hinged vertical beam, the wooden side partitions preventing any lateral movement.

Immediately above the stocks and suspended by a cord from a pulley in the roof was a rack holding four glass cylinders each containing two litres. From each of these cylinders depended a length of rubber tubing ending in a long and fairly strong hypodermic needle, and provided with a clip.

Method of Reinforcing.

Before reinforcing, the cylinders, tubing, etc., were brought directly from the steam steriliser, placed in position, and the cylinders filled with the citrated blood. The animal to be reinforced, which had previously been shaved over four small areas on the neck and quarters, was brought in and placed in position, a stable-man standing at the head and holding the head rope and another at the tail with a receptacle to catch any droppings.

The shaved areas were washed with 2% lysol, afterwards with spirit, and finally well rubbed with a pad of wool soaked in 1 in 1000 solution of perchloride of mercury.

The hypodermic needles, previously dipped in pure lysol, which acts as a lubricant and facilitates the passage through the very tough hide, were plunged deeply into the muscular tissue and the clips on the rubber tubes removed.

The cylinders of blood were raised so as to obtain a head of from two to three metres under which the blood flowed in.

In new animals the process went very quickly, but animals which had been under treatment for a long time there was often considerable difficulty, and it was necessary to change the position of the needles until a less resistant site was found. This difficulty limits the period for which an animal can be used and after 14 or 15 reinforcements the time occupied becomes so long and the process so tedious that it is almost impracticable.

After four litres have been injected—usually one litre in each of four places—the needles are withdrawn, pressure is applied with sterile wool, and a layer of collodion applied.

The arrangement of the Reinforcing Rooms and the technique was worked out by Dr. Symmers and proved most satisfactory, having the great advantage of simplicity and absence of complicated apparatus and lending itself to rapid work, a very necessary point where large volumes of blood have to be dealt with. During the year July 1904 to June 1905 a total of 2,747 animals were reinforced. This represents 10,988 litres of virulent blood, or about 35 litres a day, working six days a week.

DIFFICULTIES, ABSCESSES, ETC.

When the writer took over the Institute in July 1904 great difficulty had been met with from abscesses following the reinforcing which was then being done subcutaneously.

The strictest precautions were instituted as regards the asepsis of the virulent blood and there was an almost complete disappearance of these abscesses, but it was found impossible to get a complete absorption of the blood in many cases. This difficulty got steadily worse, the resorbing power of the sites of injection becoming apparently less and less after repeated injections.

At this time the work was being accomplished under very trying conditions, the Reinforcing Rooms being constructed of matting only and reinforcing having often to be done during sand-storms when any approach to aseptic work was quite impossible.

In December 1904 the difficulty became so great that it was decided to try intramuscular injection and the Reinforcing Rooms were rebuilt in wood with a high roof so as to obtain a greater pressure by raising the cylinders higher.

The effect of the intramuscular injections was most striking and the difficulty of abscesses and of non-absorbtion of the blood entirely disappeared.

This is very well shown in the following table. The abscesses and cases where the blood was not normally absorbed are put together as the latter cases were probably to some extent at any rate due to sepsis, and it was often impossible to say if a collection of non-absorbed blood was sterile or not.

Table showing the percentage of reinforced animals in which the blood was not normally absorbed but formed a swelling (abscesses or collection of non-absorbed blood):—

DATE.	Swellings.	REMARKS.
1904		
August	6%	Reinforcing subcutaneous.
September	6	" "
October	10	" "
November	20	" "
December	3	Reinforcing mainly intramuscular.
1905		
January	1	Reinforcing intramuscular.
February	0	" "
March...	0	" "
April	0	" "
May	0	" "
June	0	" "
July	0	" "

Normally, after subcutaneous inoculation the blood is absorbed in three to five days, but sometimes it takes a week. After intramuscular inoculation the swelling has usually disappeared in two days.

The reinforcement is generally followed by a transient rise of temperature the same evening or next morning, this is soon over and as a rule the temperature remains normal until about three days after the injection when a definite rise takes place lasting for some days and resembling that of a modified attack of Cattle Plague.

Chart 7, Plate II, shows the course of the temperature in an Egyptian bull after his seventh reinforcement. The effects of the injections in different animals and in the same animal at different times may vary enormously and it is impossible to generalise. Sometimes there is a considerable degree of fever with loss of appetite lasting over several days and sometimes practically no symptoms are observed. This irregularity has no relation to the virulence of the blood as when two animals are reinforced with the same blood one may have a severe reaction while the other shows hardly any symptoms.

As is the case with the simultaneous inoculations, the reinforcing may light up an old Texas Fever infection and so cause the death of the animal or death may occur from other causes.

During the year 1904–1905 the following deaths occurred after reinforcing :—

After first reinforcing (out of 271 animals) :—
Texas Fever 3.

After second reinforcing (out of 268 animals) :—
Texas Fever 5.
Purpura 3.

After third reinforcing (out of 82 animals) :—
Texas Fever 2.
Purpura 4.
Anemia (? cause) 1.

These were animals in which the reinforcements followed one another without an interval of bleedings as usual.

When the Institute was first started and the demand for Serum was urgent the animals were bled after one reinforcement, but later the method recommended by Koch was tried, *i.e.*, of reinforcing three times—each time with four litres of virulent blood—before the first bleeding for Serum. This, however, had to be abandoned as it was found to be too great a strain on the animals, 7 out of 82 (or nearly 9%) dying from various causes after the third reinforcement. The system of two reinforcements before bleeding was therefore adopted.

PURPURA.

A very interesting pathological condition was noted in some of the animals after reinforcing: this is the condition previously referred to as "Purpura."

About twenty cases of this were seen.

The condition was obviously definitely connected with the process of reinforcing and invariably appeared from eight to nine days after reinforcing.

Of the cases of which notes were kept no case occurred after the first reinforcement: three after the second, six after the third, two after the fourth, one after the fifth and one after the seventh. It was therefore not common after an animal had passed his fourth reinforcement.

The condition was very fatal. Of the 13 cases of which notes were kept, 10 died.

The symptoms were: passage of blood, often in large quantities, per rectum ; hæmorrhages in the mucous membranes and under the conjunctivæ, great anæmia and paleness of membranes without any rise of temperature.

Post-mortem the subcutaneous and internal fat was found to be studded with small hæmorrages which were also found under both the parietal and visceral layers of the peritoneum and in the mucous membranes of the abomasum and of the small and large intestines.

The urine did not contain blood. No parasites could be detected in the blood and cultures from the organs and sites of reinforcing in the one case which was bacteriologically examined proved negative. The cause was not discovered and the cases ceased in the spring of 1905, so that it was imposible to go further into the matter. A fuller account of this condition has been published by Mr. Rabagliati in the "Journal of Comparative Pathology" (Vol. XIX. 1906. p. 113).

BLEEDING FOR SERUM.

Date of Bleeding.

The first bleeding for Serum took place fourteen days after the last reinforcement and was followed by two subsequent bleedings at intervals of a fortnight, so that the typical course of the whole process was as follows:—

Simultaneous inoculation (1 cc. virulent blood + the necessary dose of Serum): variable interval depending on the reaction.

1st reinforcement (4 litres virulent blood): interval of 14 days.
2nd reinforcement (4 litres virulent blood): interval of 14 days.
1st bleeding (4 litres): interval of 14 days.
2nd bleeding (4 litres): interval of 14 days.
3rd bleeding (4 litres): interval of one or two days.
3rd reinforcement (4 litres virulent blood): interval of 14 days.
and so on.

Method.

The animals were selected for bleeding after their temperature-charts had been inspected and the animals themselves seen by one of the veterinary surgeons.

The bleeding took place in the Bleeding Rooms where the animal was thrown on a large straw mattress covered by a waterproof sheet. The four feet were tied together and a stable-man held the head. The neck was shaved over the jugular vein and the shaved area disinfected with lysol.

A small incision was made through the skin over the vein, which was made prominent by a pad compressing it at the root of the neck. A sharp canula furnished with a short length of rubber tubing was inserted and the blood collected in glass cylinders holding about two litres each. These cylinders were covered with muslin which was tied over the cylinder before sterilisation: just before bleeding a slit was made in the muslin to admit the rubber tube; the aperture was afterwards closed with a plug of cotton-wool.

As a rule four litres of blood were taken from each animal: this is a rather small amount, but it was found better to keep the animals in good condition and to be content with a somewhat small yield of blood rather than to risk over-bleeding them.

After removing the canula the wound was irrigated with perchloride, a catgut stitch put in and a collodion dressing applied. At first some difficulty was experienced from the bleeding wounds becoming septic, this was at the time when silk sutures were being employed and these evidently by their capillary wick-like action were the cause of the trouble, as this ceased immediately on changing to catgut.

The collodion dressing was subsequently omitted and replaced by simply dusting the wound with a mixture of powdered iodoform and boracic acid.

After being bled the animals were taken back to the stables where they were watered and received an extra ration of beans.

BLEEDING TO DEATH FOR SERUM.

Before closing the Institute a certain number of animals which were not considered fit for hard agricultural work were bled to death for Serum. This was also done in the case of animals where reinforcing had been done so often that there was great difficulty in injecting the blood owing to the fibrous thickening at the sites of inoculation. The bleeding to death took place from the carotid in the same way as the bleeding for virulent blood. A very good yield of blood was obtained and this clotted well.

The volume of blood given on bleeding to death in this way seems to be very constant, a series of 33 consecutive Egyptian animals, whose weights averaged 548 kilos., gave an average yield of 21·7 litres of blood. A second consecutive series of the same number of animals whose average weight was 540 kilos. gave an average of 21·8 litres.

This is approximately one twenty-fifth of the body weight.

The carcases of these animals were sold to the butcher as meat.

METHOD OF OBTAINING SERUM FROM BLOOD.

The cylinders after being filled with blood were placed in a cupboard in the Bleeding Room until coagulation had taken place when they were carried into the Serum Room, they were then sharply rotated to detatch the clot from the walls of the cylinders, and placed in the ice safe.

The Serum Room was a wooden building with concrete floor and double roof, the walls being double with a space of about six inches between the inner and outer walls; this space was filled with shavings. A zinc-covered table ran down the centre of the room and a row of large ice chests along two sides.

The bleeding for Serum took place daily between 9 a.m. and noon and the first decantation of Serum took place about 5 p.m. the same day, when about 12% of the total volume of the blood was obtained. The clots were then emptied out into shallow enamelled basins and put back into the ice-safe. Early next morning the Serum was again poured off the clot, this decantation yielded a further 27%.

The clots were then cut into thin slices and suspended in the ice-safe in bags of mosquito-netting until next day when a further yield of about 6% was obtained. Making a total yield of 45%.

Each decantation was placed separately in sterile bottles and sent down in ice to the Public Health Department in Cairo where it was centrifugalised the same day and bottled, with the addition of 0·5% carbolic acid and glycerine (Ehrlich's mixture).

The yields stated above are from the figures obtained over a period of six months. The amounts obtained vary enormously in various animals and in the same animals at different times, in some cases there was a very imperfect clotting and in others a good clot but hardly any separation of Serum.

The method of turning the clots out into basins and again of cutting them up and allowing them to drain was instituted by Dr. Symmers and was found to greatly increase the yield of Serum. The Serum from the cut up clot was somewhat hæmoglobin stained but it was kept apart from the rest and after centrifuging was carbolised and issued for immediate use.

The method appears somewhat rough and ready and certainly leaves much to be desired from a bacteriological point of view. It is, however, simple and can be carried out by unskilled assistants and gives a very good yield of Serum. In practice it was found to work admirably.

The Serum after centrifuging and the addition of carbolic was put up in bottles containing about 225 cc., corked, sealed, and kept in a special room in the cold storage at Cairo.

TESTING STRENGTH OF SERUM.

In order to be able to recommend a suitable dose of the Serum for its practical use on a large scale it is obviously necessary that its strength should be known with, at any rate, some degree of accuracy. This is a question which presents considerable difficulties.

As the tests can only be made on cattle, and as they require a considerable number of animals in order to obtain trustworthy results, each test involves a somewhat large expense. For this reason it is quite impossible to test the sera of individual animals from time to time during their immunisation as is usually done in the case of animals providing antitoxic sera (*e.g.*, Diphtheria and Tetanus sera), but tests can only be made on large batches of the mixed sera of many animals.

For practical purposes, however, these are sufficient.

The first tests made by the writer were on Cyprus cattle but did not give good results, chiefly from the fact that at the time of year when the tests were made these animals are very liable to contract Egyptian Fever, and this, complicating the Cattle Plague reaction, often caused death. Moreover, they are very much more susceptible to Cattle Plague than the Egyptian animals and until comparative tests had been made, the dose necessary for a Cyprus animal gave no clue to the dose required for Egyptian animals.

On Egyptian Animals.

It was therefore decided to determine as exactly as possible the necessary dose for Egyptian animals. Here again a difficulty arose, for as at the time the disease had been in the country for some time it was impossible to obtain animals known with certainty not to have had the disease. This difficulty could only be met by using a large number of animals, this was accordingly done. A batch of serum (Lot 35) which was believed to be somewhat weak was selected and a preliminary test made on ten Egyptian animals. Of three of these which each received 20 cc. per 600 lbs. body-weight one died but was found to have markedly tubercular bronchial glands, the other two receiving the same dose recovered. Those receiving larger doses all recovered. From this preliminary test it appeared that the correct dose was somewhere near 20 cc. per 600 lbs.

A more complete test was then made on 40 Egyptian cattle which were selected by the Veterinary Department as far as possible from villages which were supposed to have escaped the disease. A special stable was built for the test so that the animals were kept under confortable conditions. Ten of the animals were kept as controls and inoculated with virulent blood only, the remainder were inoculated with 1 cc. of virulent blood and varying doses of Serum. This test is shown in Table No. 1.

It will be seen from the tables that of the control animals receiving virulent blood only, 5 (*i.e.*, 50%) had no reaction and 3 (*i.e.*, 33% only) died of typical Cattle Plague. One animal died of Cattle Plague 24 days after inoculation but cannot fairly be included, as on post-mortem examination he was found to be suffering from advanced tuberculosis.

The result in these controls shows the absolute necessity of working with a large number of animals.

Of the 30 animals which received blood and serum 15 showed no reaction. The largest dose of Serum with which death took place was 20 cc. per 600 lbs., 4 animals which received 23 cc. per 600 lbs. recovering.

The necessary dose of the Serum therefore to protect an Egyptian animal against a simultaneous inoculation of 1 cc. of virulent blood was taken as 23 cc. per 600 lbs.

On Cyprus Cattle.

The same Serum (Lot 35) was now tested on Cyprus cattle.

This test is shown in Table No. 2.

It will be seen that for these the necessary dose was more than 100 cc. and less than 150 cc. per 600 lbs.

RELATIVE SUSCEPTIBILITY OF CYPRUS AND EGYPTIAN ANIMALS.

The amount of the Serum required for a Cyprus animal is therefore approximatively six times that required for an Egyptian animal.

As the trouble with Egyptian Fever in the imported Cyprus cattle only occurred during the tick season in summer, during the rest of the year Cyprus cattle could be employed for the tests; and once the ratio of the amounts of Serum required in the two respective races was known, these animals were used. In this way the testing was rendered very much easier as owing to their high susceptibility reliable results could be obtained from a comparatively small number of Cyprus animals, whereas, in the case of Egyptian animals one had always to contend with the difficulty that a very large percentage of the animals showed a greater or less degree of immunity.

This partial immunity of the Egyptian animals is interesting as it is improbable that it is, at any rate, wholly due to the animals having previously had the disease.

This was shown by some of the earlier experiments of Dr. Symmers. In one of these an Egyptian bull was inoculated in July 1903 (*i.e.*, very early in the epizootic) with 3 cc. of virulent blood without any Serum. He developed a typical severe attack of Cattle Plague but recovered.

Had the immunity been due to a previous attack of the disease it is highly improbable that such a severe reaction would have followed the inoculation of 3 cc. of virulent blood. Moreover, in the simultaneous inoculations made early in the epidemic it was noticed that many of the animals showed a considerable degree of immunity.

The Serum (Lot 35) used in the above test was chosen as being probably one of the weakest batches, as it contained the Serum of a considerable number of animals which were in a rather early stage of their immunisation. As it was impossible to test all the batches this weak one was intentionally selected in order to determine the minimum dose which could be safely employed in practice.

INCREASE IN STRENGTH OF SERUM AFTER REPEATED REINFORCING.

The strength of the Serum appears to increase after the animals have been under treatment for some time: a test was made of the mixed sera of three bulls which had been in continuous use for over a year. It was found that this protected Cyprus animals at a dose of 50 cc. per 600 lbs. and it was not tested higher. This corresponds to a dose of about 8 cc. per 600 lbs. for Egyptian animals and is at least three times the strength of the Serum used in the large test given above (Lot 35).

This experiment should be repeated, but the difference is so great that there seems to be little doubt that the strength of the serum increases after repeated reinforcements.

Unfortunately, owing to the great cost of these tests, this, with many similar interesting points, could not be continued.

In all tests it is most important that the cattle are well housed and cared for, as during the height of the reaction they are very susceptible to cold. When their temperature is high they naturally suffer from thirst and require a liberal supply of water: this is a point that requires attention as the fellahin appear to have a prejudice against giving much water to cattle suffering from the disease.

REMARKS ON SOME DIFFICULTIES MET WITH IN THE PROCESS OF SERUM PRODUCTION.

The question of abscesses, non-absorption of blood, Texas and Egyptian Fevers has been already referred to.

Foot and Mouth Disease.

The period of quarantine imposed on all animals entering the Institute acted as a very efficient barrier to the entrance of any epizootic disease, and the only difficulty of this kind met with was in the case of Foot and Mouth Disease which on three occasions gained entrance to the compound. This was traced, at any rate on two occasions, to infected forage.

The second outbreak began about six months after the termination of the first. When this second epidemic began the compound contained about 200 old animals, *i.e.*, animals which had been in the Institute during the first outbreak, and about an equal number of new animals.

Altogether 96 cattle contracted the disease (85 of these being new animals), whilst of the old animals only 11 were attacked. The incidence rate was thus about eight times greater in the new than in the old animals, which is interesting as showing that more than six months after an attack of this disease there is still a comparatively high degree of immunity.

In the third outbreak, which began about a month after the end of the second, 30 animals were attacked—19 new and 11 old.

These outbreaks were met by inspecting the animals in the compound twice a day, any animal found with the disease being at once removed to the Sick Lines, and the stable from which he was taken being disinfected, *i.e.*, the floors soaked, the mangers scrubbed with carbolic and the whole stable whitewashed. The stable-man who had been grooming him was made to wash himself thoroughly and his clothes were taken away and disinfected.

Arrangements were made for watering the animals from suspected stables at special troughs.

Before recovered animals were brought back from the Sick Lines into the compound they were washed all over with 2% lysol solution, and during the process of washing stood in a shallow tank containing enough of the solution to cover the fetlocks. In this way any infectious material adhering to the feet was got rid of and not carried into the compound. This tank, or "foot-bath," was made of concrete and had sloping ends so that the cattle could be walked in and out.

This disease, owing to its very highly infectious nature, is most difficult to deal with, and in spite of all the measures taken cases kept on cropping up. The distribution of these cases was so irregular that suspicion was thrown on the saices, and it was found that some of the stable-men looking after the animals in the Sick Lines were in the habit of paying visits to their friends in the compound; when this was stopped no further cases occurred.

At the time of the first epizootic of Foot and Mouth Disease in the Serum Institute Foot and Mouth Disease was said to have broken out in several villages shortly after the animals in these villages had been inoculated with Serum and some of the Inspectors of the Veterinary Department who were using the Serum were of the opinion that this was the cause of these outbreaks.

In view of the fact that the Serum as sent out contained 0·5% carbolic this does not appear probable, but with our present small knowledge of the nature of the causal agent of the disease one would hesitate before pronouncing it impossible.

The only other contagious disease met with was Psoroptic Mange: this was not severe and yielded readily to treatment.

During the first summer after the Institute was started ticks were very troublesome. These were *Hyalomma Aegyptium* and *Rhipicephalus Annulatus*. During this summer the cattle were cleaned every day and all ticks destroyed. The stables were disinfected from time to time and during the following summer (1905) the result of these measures was most striking, it being quite uncommon to find a single tick on any of the cattle.

This must have been a very great comfort to the animals.

GENERAL REMARKS ON PROCESS AND METHODS.

The process described above gave on the whole very good results. The large majority of the cattle kept in excellent condition throughout the treatment: any which were seen to be losing condition or not thriving were killed and were almost invariably found to be tubercular. It is interesting that so many of the Egyptian cattle, which were fine healthy looking animals, were tubercular, whilst in Cyprus cattle, which were miserably poor creatures, tubercle was exceedingly rare; indeed out of more than a thousand which were examined only one case of tubercle was found.

When the Institute was closed 200 cattle were selected and sent to a large estate where they were put to hard agricultural work, ploughing, etc. These animals a year later were reported to have worked well in spite of the fact that some of them had previously been producing Serum for a year and a half, during which period they had been regularly bled four litres every fortnight.

Arrangements were made by the Government with the owners of the estate in question that in case of necessity these animals would be returned to the Department, so that in the event of a serious outbreak of Cattle Plague the production of Serum could be resumed without delay.

NUMBER OF ANIMALS REINFORCED, ETC.

From July 1904 to the close of the Institute in September 1905, over which period exact figures are available, the following are the numbers of animals treated and the quantity of Serum prepared:—

Reinforced with 4 litres of virulent blood	3,193 animals.
Bled for Serum	8,713 „
Cyprus animals bled to death	1,098 „
Serum obtained	17,504 litres.

COST OF SERUM.

During the whole period in which the Serum Institute was working (*viz.*, September 1903 to September 1905) a total of 20,916 litres, or 418,320 doses of 50 cc. each of Serum were manufactured.

The total cost of the Institute was:—

	L.E.	Mill.
Equipment	2,177	829
Drugs and Dressings	454	810
Surgical Instruments, etc.	651	551
Centrifuge and Fittings	800	000
Forage	12,010	540
Animals purchased	30,117	071
Food of Staff	2,022	761
Salaries and Wages	5,673	747
Buildings, Rails, etc.	2,052	012
Water	19	242
Storage of Serum	21	937
Petty Expenses	41	285
	56,042	785
Deduct Animals and Carcases sold	14,614	572
Total...	41,428	213

This total represents fairly accurately the actual cost. Certain buildings, with the centrifuge, some of the equipment, instruments, etc., have not been struck off as these are retained by the Public Health Department in view of the possibility of another epidemic.

418,320 doses of Serum (of 50 cc. each) were therefore made at a cost of L.E. 41,428, making the cost per dose 9·9 piastres, or two shillings.

SCALE OF FORAGE.

Proposed by the Chief Veterinary Inspector, for the Serum Institute animals.

Daily ration per animal.

Tibn	7 kilos.
Beans	4 „
Drees	2 „

or

Tibn	7 kilos.
Beans	3½ „
Cotton cake	½ kilo.

To replace drees 7½ kilos. bersîm or green durra for one kilo. of drees.

Special rations for sick animals to be prescribed by the Veterinary Inspector in charge.

Animals suffering from abscesses to receive the ordinary rations.

Animals suffering from fever after being reinforced to receive :—

Green durra or bersîm	10 kilos.
Drees	2½ „
Beans	2 „
Tibn	2½ „

APPENDICES.

APPENDIX 1.*

SOME EXPERIMENTS ON THE FILTRATION OF CATTLE PLAGUE BLOOD.

The outbreak of Cattle Plague in Egypt and the establishment of the Serum Institute at Abbassîa offered a suitable opportunity for investigating some points concerning the nature of the causal agent of the disease, and especially the important question whether the virus is, or is not, capable of traversing a filter candle. On this point the literature is strangely contradictory.

Semmer (*Deutsche Zeitschrift f. Tiermedicin, und vergleichende Pathologie.* Bd. 20, Heft 1, p. 32.) found that infective materials after passage through a Chamberland filter were non-virulent.

Nencki, Sieber and Wyznikiewicz (*Centralblatt f. Bakt.* Abt. 1, Bd. 13, p. 535.) found that the filtrates from both Berkefeld and Chamberland filters were non-virulent.

Kolle and Turner (*Zeitschrift f. Hyg.* Bd. 28, p. 361.) state that the microbe does not pass through Pasteur, Chamberland, or Berkefeld filters, and that when virulent blood is passed either slowly or quickly through such filters, the filtrate, even in large quantities, is not infectious, whilst the material remaining on the filters is highly so.

To eliminate the possibility of this result being caused by the microbe being an obligatory intracorpuscular parasite and so never being really free in the blood, the corpuscles were hæmolysed by the addition of 0·2% sodium chloride solution, and it was found that this had no influence on the result.

Kolle and Turner are of the opinion that the parasite is not so small as to be invisible to modern lenses, but at the same time hold that the probability of seeing it is very small, as it must be at any rate less that an influenza bacillus.

Kolle (*Zeitschrift f. Hyg.* 1899. Bd. 30, p. 36.) showed that if defibrinated Cattle Plague blood—either hæmolysed or not—be centrifuged at a speed of from 2,900 to 3,000 revolutions per minute for 20 to 30 minutes, the parasite is entirely driven down with the deposit, which is highly infectious, the supernatant fluid being quite free from infection.

Nicolle (*Annales Pasteur.* T. 13, p. 323.) agrees completely with the above results. He says: "Si l'on filtre le sang, défibriné et étendu au dixième, sur le filtre Chamberland ou sur la bougie Berkefeld, le liquide se montre inoffensif, mais il ne vaccine pas"; but in a subsequent paper with Adil Bey (*Annales Pasteur.* 1902. T. 16, p. 56.) the authors state that the virus is capable of traversing Berkefeld and also with greater difficulty Chamberland F. candles, but only under certain conditions. They conclude that the microbe is commonly intraleucocytic.

Yersin (*Annales Pasteur.* 1904. T. 18, p. 429.) working with fluid obtained by intraperitoneal lavage in animals suffering from the disease, found that the virus passed through Chamberland filters, mark F, but was stopped by mark B.

Memmo, Martoglio and Adani (*Annali d'Igiene Sperimentale.* 1904. Vol. 14, p. 256.) working in Eritrea, come to the conclusion that the virus passes the Berkefeld candle but is stopped by the Chamberland F.

There is thus a very wide discrepancy in the results of the different workers. Semmer, Nencki Sieber and Wyznikiewicz, and Kolle and Turner, all agree that the virus is not a filter-passer and the last-named authors find that it is not only incapable of passing through an ordinary Berkefeld filter but is comparatively easily removed from suspension by centrifugalisation. Memmo, Martoglio and Adani, on the other hand, find that it passes the Berkefeld candle but not the Chamberland F, while Nicolle and Yersin find that it passes a Chamberland F.

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* This was Published in the "Journal of Hygiene," Vol. VII, No. 4. July, 1907.

Filtration experiments, in the case of a disease so highly infectious as Cattle Plague, are surrounded by many possibilities of error. Apart from the question of a flaw or other weakness in the filter candle itself, which can be more or less easily controlled, there is the great danger of accidental infection of the inoculated animals. Unless the strictest precautions are taken this may take place through channels which would hardly be suspected by persons who had not had an actual acquaintance with the disease.

For this reason one is inclined to place much more reliance on a few negative results carried out under strict conditions, than on a larger number of experiments giving positive results.

Nicolle and Yersin, who both found that the virus passes a Chamberland filter, are by no means in agreement as to the ease with which this takes place. Nicolle says: "On réussit *rarement* et encore n'arrive-t-on qu'à vacciner les animaux"; whilst Yersin finds that "Le virus traverse *constamment* la bougie F." The latter author unfortunately only quotes one experiment, and in this does not give the temperature chart of the animal inoculated, so that it is impossible to form an opinion. Nicolle gives complete details of three animals inoculated with Berkefeld filtrates, two of these remained well but were afterwards found to be immune, the third contracting a fatal attack of the disease after a long incubation period. The initial rise of temperature took place on the morning of the eighth day, and Nicolle attributes this long incubation period to the small number of germs inoculated and to their dilution. This explanation is, however, somewhat difficult to accept, and is certainly not in agreement with the experience at Abbassîa, where it was found that with wery small doses of virulent blood (down to 1/500 cc.) the temperature rose after exactly the same incubation period as in the case of large doses.

This constancy of the incubation period is most definite, and, as was pointed out in the description of the disease in Cyprus animals (p. 16), out of 637 animals inoculated with virulent blood in no case did the initial rise of temperature occur later than the fifth day. One is therefore inclined to regard the animal referred to by Nicolle as having contracted the disease accidentally at some date subsequent to the injection of the filtrate.

This accidental infection is by no means easy to avoid, as was realised in the first filtration experiment made at Abbassîa.

In this case two Cyprus bulls were inoculated with 200 cc. of the filtrate of virulent blood diluted to 1 in 4 with saline before filtration. The animals were placed ouside the compound in a separate stable and ordinary precautions were taken to avoid infection, but they were attended to by members of the staff of the Institute. The temperatures of both animals began to rise on the fifth day after the injection and both animals developed typical Cattle Plague. As it is exceedingly rarely, if ever, that the incubation period in Cyprus animals lasts so long as this after subcutaneous inoculation, while after infection by the mucous membranes (*e.g.*, smearing the nostrils with infective materials) this period is the rule, it was almost certain that these animals had in some way become infected naturally—probably during handling in the course of injection. Further experiments were therefore postponed until they could be done under thoroughly strict conditions.

A very striking point in Nicolle's results is the number of cases in which he obtained "vaccinating filtrates," *i.e.*, the animal remained well after the injection of the filtrate, and when tested with virulent blood, from ten to fifteen days later, resisted infection. This in itself is a most interesting fact and it is a pity that the temperature charts are not given.

It would be interesting to know if the animals employed (animals from Anatolia, Crimea, etc.) are not sometimes immune.

In an earlier paper he states that he has tested the comparative susceptibility of the various races most commonly found in Turkey, and that the Crimean, Anatolian, and Egyptian animals are very susceptible, and that in them death constantly takes place after the injection of virulent blood. For the Egyptian animals, in Egypt at any rate, we know that this is by no means the case.

In repeating these experiments on the filtration of virulent blood the greatest precautions were taken to obviate all possible chances of accidental infection.

The Cyprus cattle which were used remained in the stables where the non-infected Cyprus cattle for the Institute were kept, and so were together with a considerable number of clean Cyprus animals which served as controls.

These stables were about a mile from the Institute and had an entirely different staff which had no connection with that of the Institute.

Virulent blood for the experiment was defibrinated by whipping, diluted with four times its volume of 0·8 % saline and divided into two parts. One part was passed through a large Berkefeld filter which was somewhat close-pored and the filtration was done as slowly as possible.

The other part was filtered as rapidly as possible through a large and very porous Berkefeld filter, and in this case ran through very rapidly. Filtration was carried out by suction from an ordinary laboratory water-pump, and the difference in pressure was therefore less than one atmosphere.

In both cases a culture of an exceedingly small bacillus was mixed with the blood before filtration, but did not pas the filter.

Fifty cc. of each of the filtrates was injected subcutaneously into two Cyprus animals, care being taken that no one who had anything to do with Cattle Plague should come in contact with the animals. The temperatures of all the four cattle remained normal—except one which had a mild attack of red-water—until ten days later when they were each inoculated with 10 cc. of virulent blood and in each case developed typical Cattle Plague, the initial rise of temperature occurring in two animals on the third day and in the other two on the fourth. All the four animals were bled to death for virulent blood, and the diagnosis of Cattle Plague verified by post-mortem examination.

Three control animals inoculated with 1/5 cc. of the virulent blood before it was filtered developed the disease typically, the initial rise of temperature occurring in two of the animals on the third and in the other on the fourth day.

The temperature charts of the four cattle which received the filtrate are given (Charts 8, 9, 10 and 11, Plate III).

This experiment shows that highly virulent Cattle Plague blood when diluted to five times its volume with saline is rendered absolutely non-virulent even by rapid filtration through a porous Berkefeld candle. This being the case it appears obvious that the microbe must be unable to traverse the much closer-grained Chamberland candle, but it was thought interesting to try an experiment to establish this.

With this idea, about 40 cc. of citrated virulent blood was introduced by means of a pipette into the interior of a sterilised Chamberland filter F. The greatest care being taken to keep the filter aseptic and to avoid touching the neck with the virulent blood. A sterile cork was then placed in the neck of the candle which was finally sealed with sealing wax. The filter containing the virulent blood was then introduced with all surgical precautions into the peritoneal cavity of a Cyprus animal through a small incision in the abdominal wall which was afterwards very carefully and thoroughly closed by sutures.

The operation succeeded well and was followed by practically no rise of temperature. The wound healed by first intention, and the animal remained perfectly well for thirteen days, when it was tested by an injection of virulent blood. This gave rise to a typical attack of the disease, death occurring eight days after the injection of virulent blood, showing that not only did the virus fail to pass the filter but that no immunity whatever resulted.

The chart of this animal is given (Chart 12, Plate III).

Reviewing the work which has been published on this question and the results of the experiments cited above, one is led to the conclusion that the evidence in favour of the active argent of Cattle Plague being a filter-passer is at present quite inadequate.

APPENDIX 2.

ON THE SUSCEPTIBILITY OF THE EGYPTIAN BUFFALO OR "GAMOOS" TO EXPERIMENTAL INOCULATION WITH CATTLE PLAGUE.

The general experience in Egypt is that, while the gamoos may contract Cattle Plague and even die of the disease, this animal is very much less susceptible than the cattle of the country.

Very often in affected villages the buffalos apparently escape altogether, and if they are affected they commonly have a benign form of the disease, though at times very acute cases are said to occur.

The Cattle Plague death rate in buffalos is not easily obtainable, as the question is complicated by "Barbone," an acute hæmorrhagic septicæmia which is present in the country in an enzootic form. This causes a large mortality amongst buffalos, and during the Cattle Plague epidemic undoubtedly a large number of cases of this disease were included in the Cattle Plague statistics.

With a view to obtaining some definite facts as to the susceptibility of the buffalo to Cattle Plague a certain number of these animals were brought up to the Serum Institute, where they could be carefully observed, and were inoculated with virulent blood.

In the first experiment four full-grown gamoos cows were inoculated subcutaneously, one receiving 5 cc. and the remaining three 10 cc. each of virulent blood.

No symptoms of any kind were observed, and the temperatures of all four animals remained normal.

As it was impossible to exclude the possibility of these animals having already suffered from an attack of the disease, further experiments were made on young gamoos calves. Nine of these, whoses ages varied from about four to six months, were inoculated with varying quantities of virulent blood (from 1/10 cc. to 350 cc.).

This blood was in all cases tested by experiments on control Cyprus cattle and was found to be highly virulent.

The temperature charts of these nine calves are annexed (Charts 13, 14, 15, 16, 17, 18, 19, 20, 21, Plate IV), and it will be seen that in every case there was some rise of temperature; generally after an incubation period of from three to five days. In some of the calves this rise of temperature was very small, but in others it was quite pronounced.

One animal (Chart 16, Plate IV) died ten days after inoculation. Post-mortem examination showed a severe gastro-enteritis with bright green bile. Some blood was taken from the heart and 2 cc. of this inoculated into a Cyprus bull, which developed a typical attack of Cattle Plague and died nine days after inoculation. This renders it highly probable that the calf in question actually had the disease, though it is possible that the Cattle Plague virus had merely remained in the blood of the gamoos without multiplication and so had been transferred to the Cyprus animal. The definitive rise of temperature following the injection of virulent blood in all the calves, however, points very strongly to their having been infected, more particularly as the rise of temperature appears to bear no relation to the amount of virulent blood injected.

It is an interesting fact that, with the exception of the animal which died, none of the nine calves showed any symptoms whatever beyond the rise of temperature, and continued to feed well even when their temperature was somewhat high.

Judging from the experiment on the calf which died and whose blood was found after death to be virulent, it is highly probable that the other animals, at any rate during the period of pyrexia, were also infectious and capable of infecting other animals.

From a practical point of view this is of the greatest importance, as it seems not unlikely that, during an epidemic, buffalos may contract the disease in a very mild form, and while apparently healthy may in reality be highly infectious and may play a considerable role in maintaining and spreading the infection.

TABLE 1.

TESTS OF SERUM (LOT 35) ON EGYPTIAN CATTLE.

Animal.	Weight in kilos.	Virulent Blood.	Serum, Actual Dose.	Serum per 600 lbs.	REMARKS.
No.		cc.	cc.	cc.	
2	482	1	—	—	*Died* (9 days).
7	445	1	—	—	*Died* (24 days). Advanced tuberculosis.
14	450	1	—	—	*Died* (9 days).
17	459	1	—	—	No reaction.
19	309	1	—	—	Recovered. Good reaction.
22	265	1	—	—	*Died* (9 days).
28	484	1	—	—	No reaction.
32	388	1	—	—	No reaction.
33	452	1	—	—	No reaction.
41	427	1	—	—	No reaction.
1	575	1	33	15	Recovered. Good reaction.
3	515	1	28	15	Recovered. Severe reaction.
4	455	1	24	14	No reaction.
5	450	1	24	14	No reaction.
6	381	1	18	12	*Died* (14 days).
25	558	1	31	15	*Died* (12 days).
26	525	1	39	20	*Died* (11 days).
27	566	1	42	20	No reaction.
29	387	1	28	20	Recovered. Good reaction.
30	458	1	34	20	Recovered. Very slight reaction.
8	387	1	33	23	No reaction.
9	420	1	37	23	No reaction.
10	457	1	41	24	No reaction.
11	415	1	37	23	Recovered. Good reaction.
12	390	1	34	23	Recovered. Good reaction.
13	409	1	35	23	No reaction.
31	427	1	48	30	Recovered. Good reaction.
34	452	1	51	30	Recovered. Good reaction.
35	513	1	57	30	Recovered. Good reaction.
36	485	1	54	30	No reaction.
15	478	1	60	34	Recovered. Good reaction.
16	483	1	62	34	No reaction.
18	459	1	58	34	No reaction.
20	670	1	90	36	Recovered. Very slight reaction.
23	620	1	82	35	No reaction.
24	574	1	76	35	Recovered. Late reaction.
37	475	1	70	40	Recovered. Very slight reaction.
38	452	1	68	40	No reaction.
39	450	1	68	40	No reaction.
40	463	1	70	40	No reaction.

TABLE 2.

TESTS OF SERUM (LOT 35) ON CYPRUS CATTLE.

Animal.	Weight in kilos.	Virulent Blood.	Serum, Actual Dose.	Serum per 600 lbs.	REMARKS.
No.		cc.	cc.	cc.	
559	445	1	165	100	*Died* (9 days).
560	343	1	125	100	*Died* (9 days).
593	405	1	150	100	*Died* (9 days).
594	400	1	150	100	*Died* (33 days).
561	390	1	217	150	Recovered. Good reaction.
562	247	1	247	150	Recovered. Good reaction.
563	370	1	270	200	Recovered. Good reaction.
564	428	1	310	200	Recovered. Good reaction.

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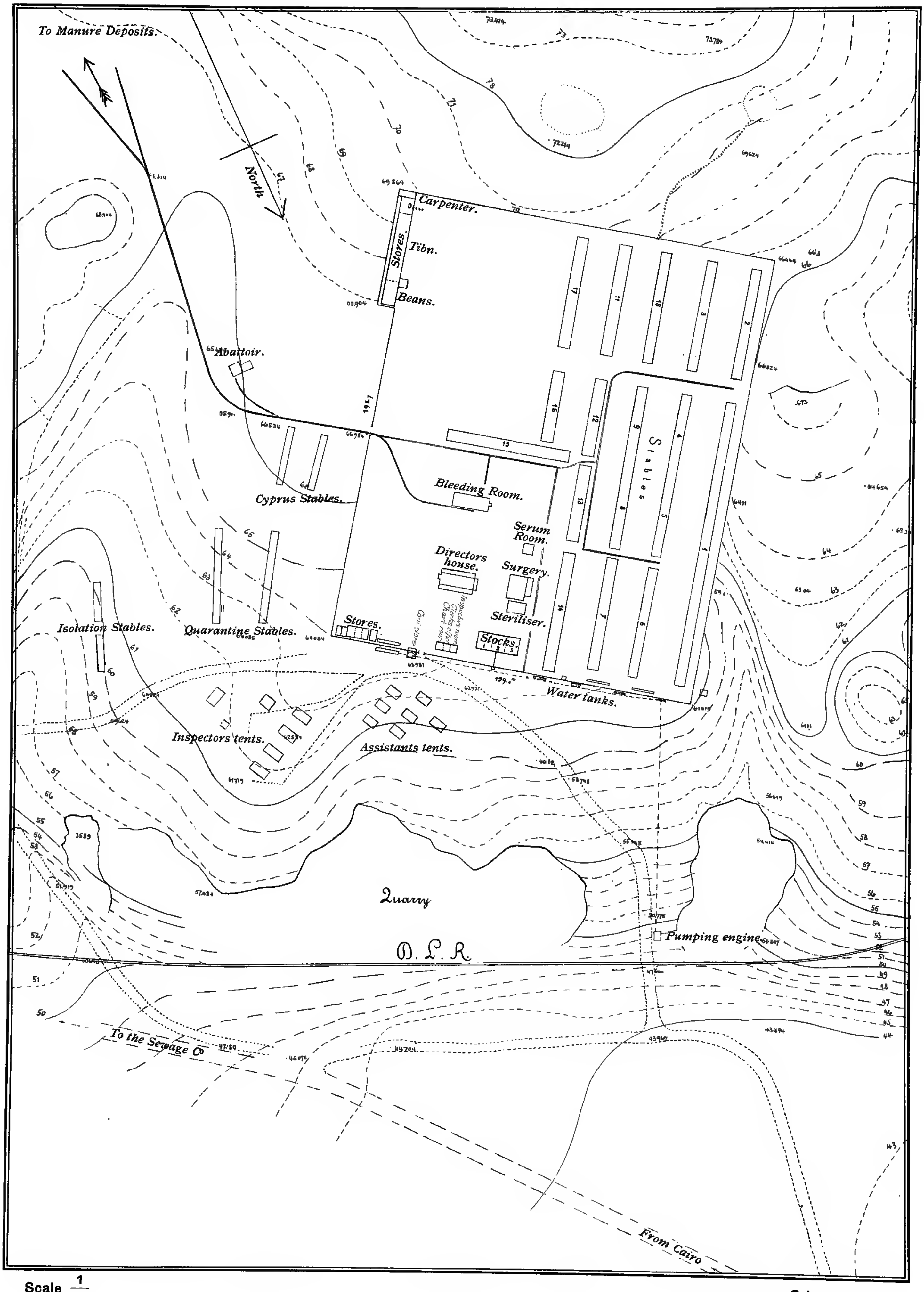


Scale $\frac{1}{400}$

von Grimm P. W. D.

Serum-Institute Abbassieh
1905.

PLATE I.

CHART 1.

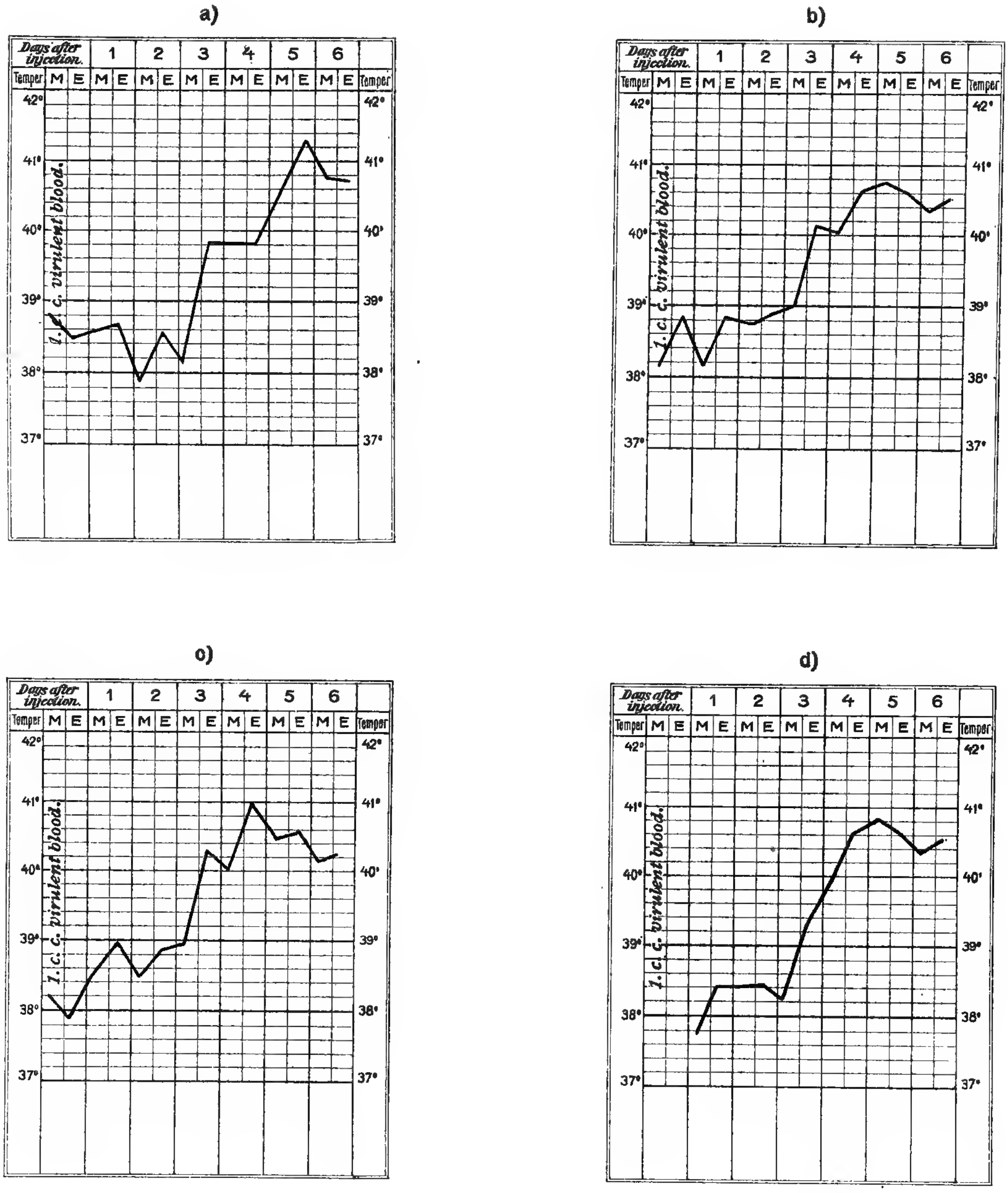

a) b) c) d)

CHART 2.

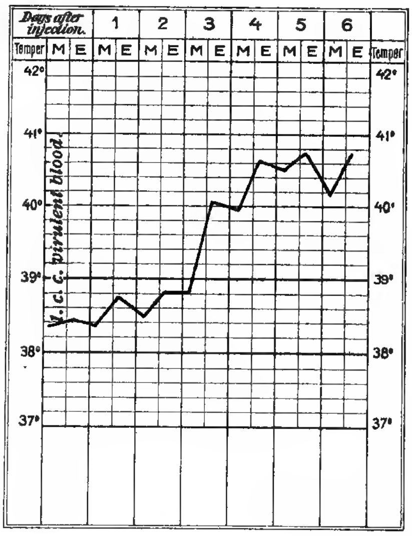


CHART 3

Experimental Cattle Plague in Egyptian Bullock (Weight 266 kilos age 4 years).

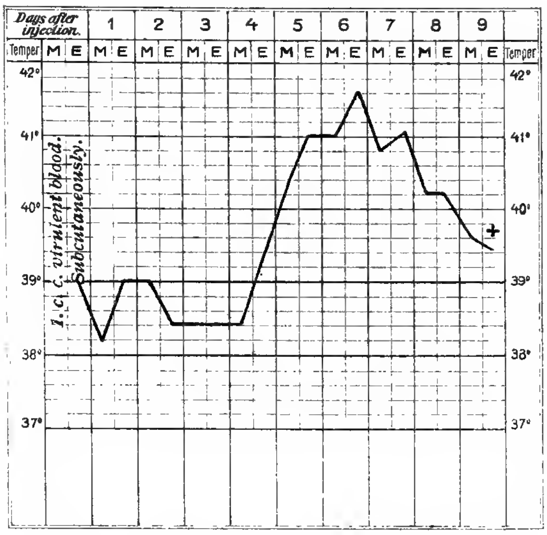


CHART 4.

Experimental Cattle Plague in Egyptian Bullock (Weight 482 kilos age 4 years).

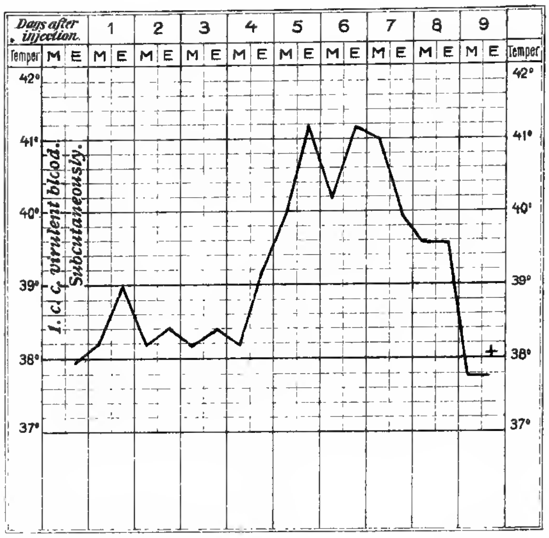


CHART 5.

Simultaneous Inoculation. Egyptian Bullock (23 c. c. Serum per 600 lbs).

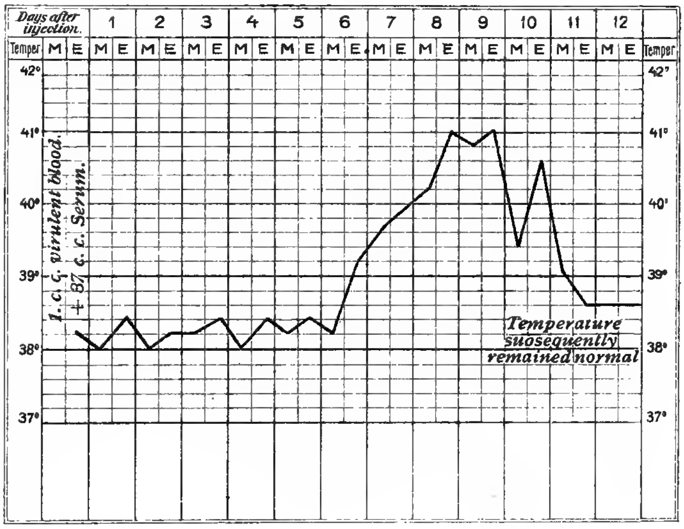


CHART 6.

Simultaneous Inoculation. Egyptian Bullock (20 c. c. Serum per 600 lbs).

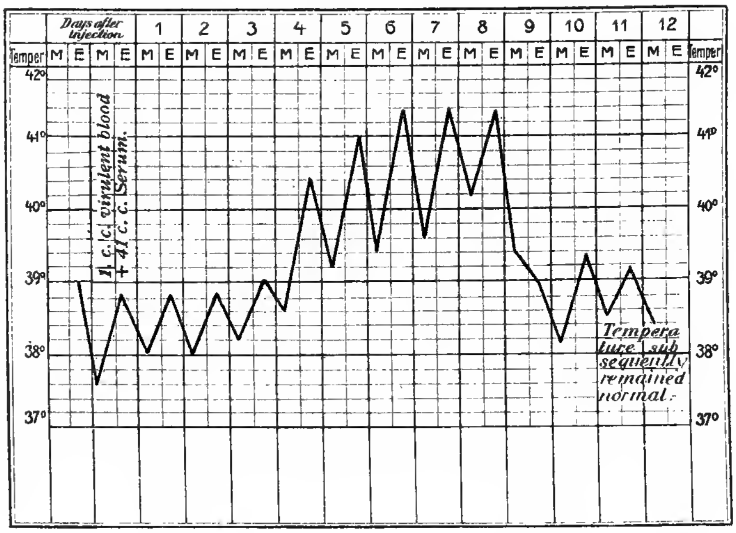
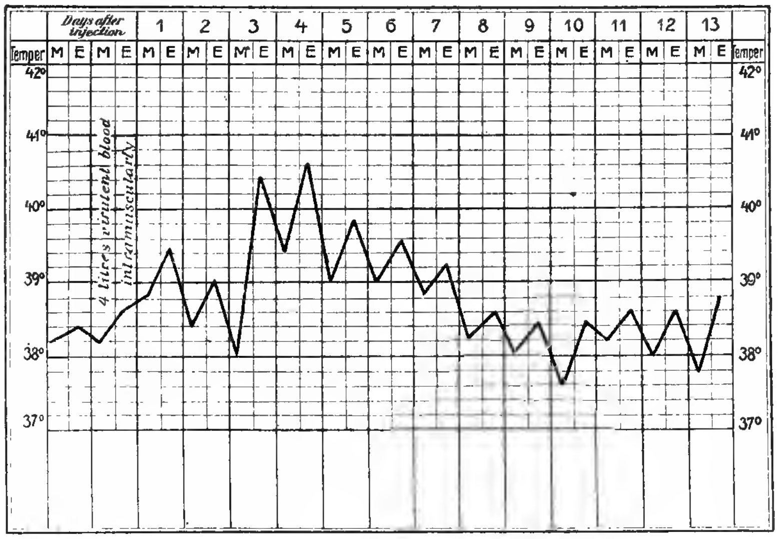


CHART 7.

Reinforcement. Egyptian Bullock. (7th time of reinforcing).



Filtration Experiments.

CHART 8.

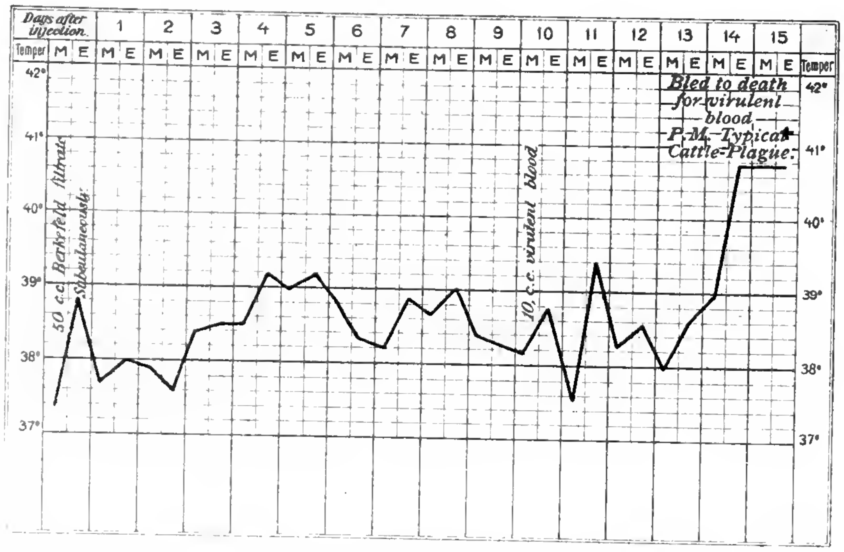


CHART 9.

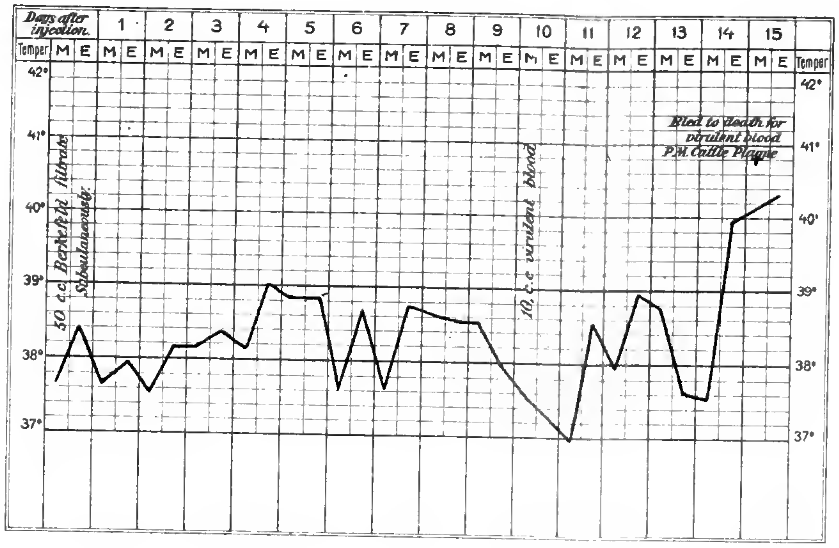


CHART 10.

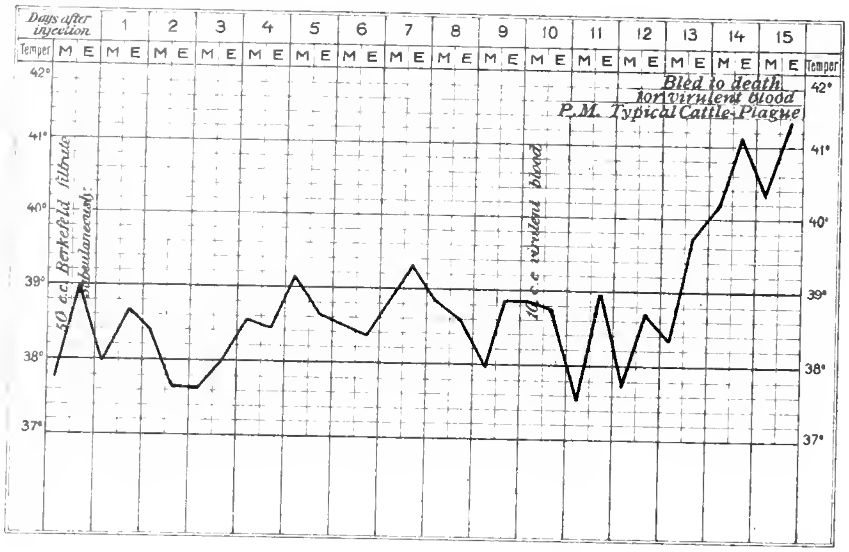


CHART 11.

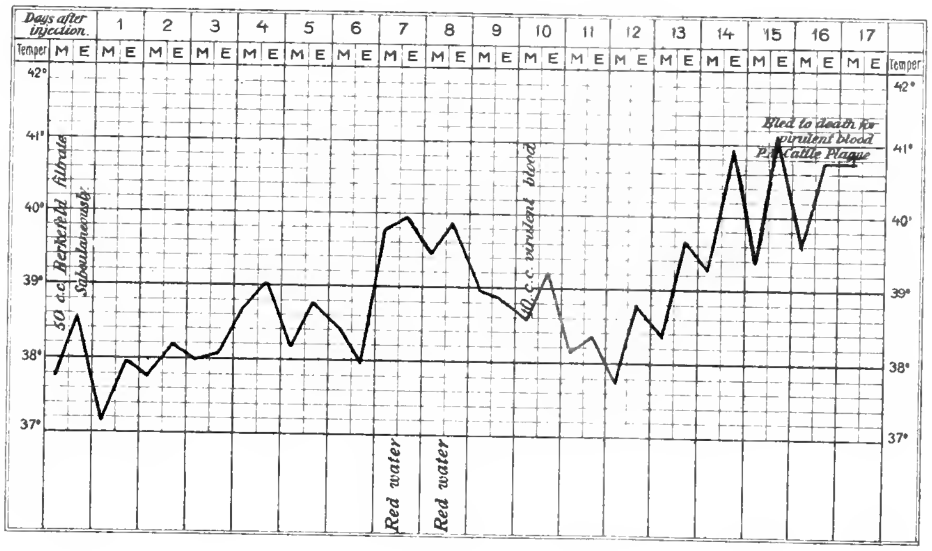
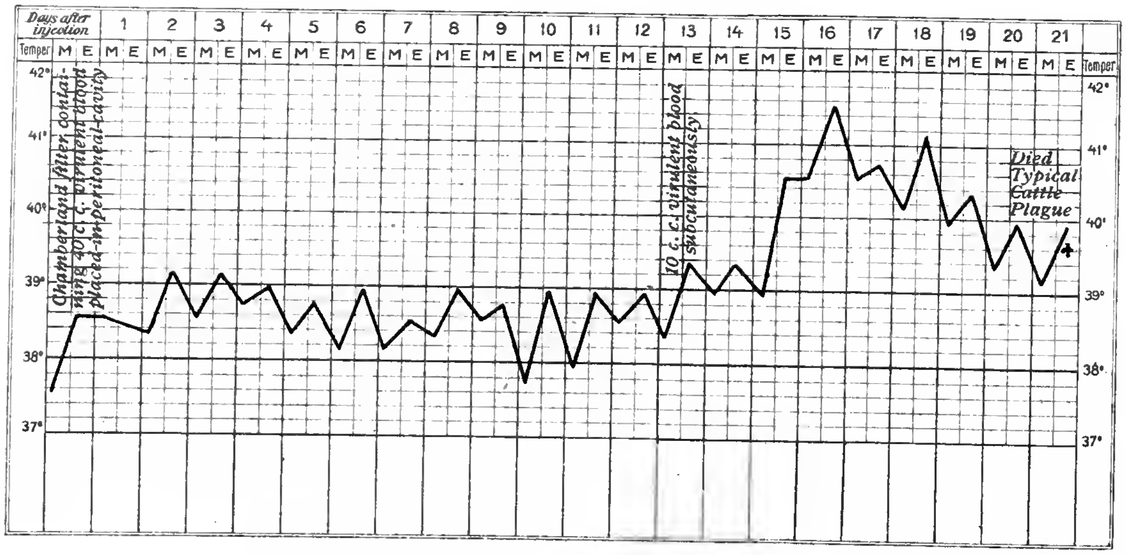


CHART 12.



Inoculation of Egyptian Buffalo with virulent blood.

CHART 13.

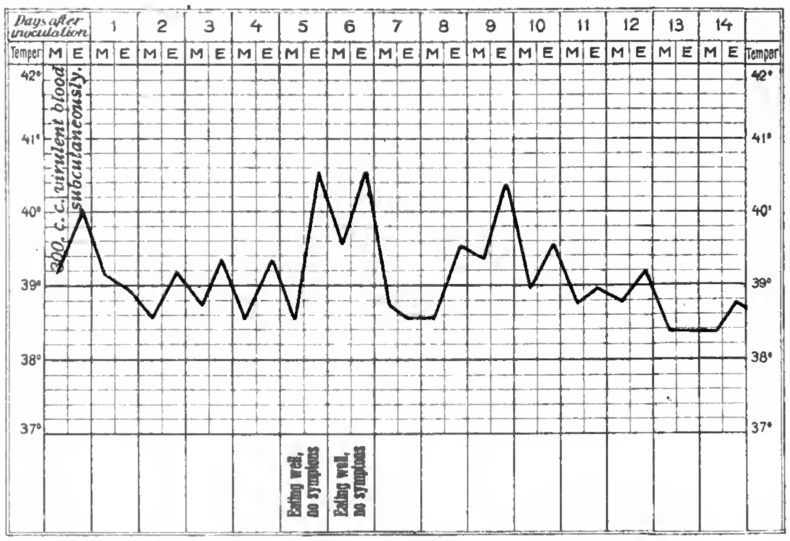


CHART 14.

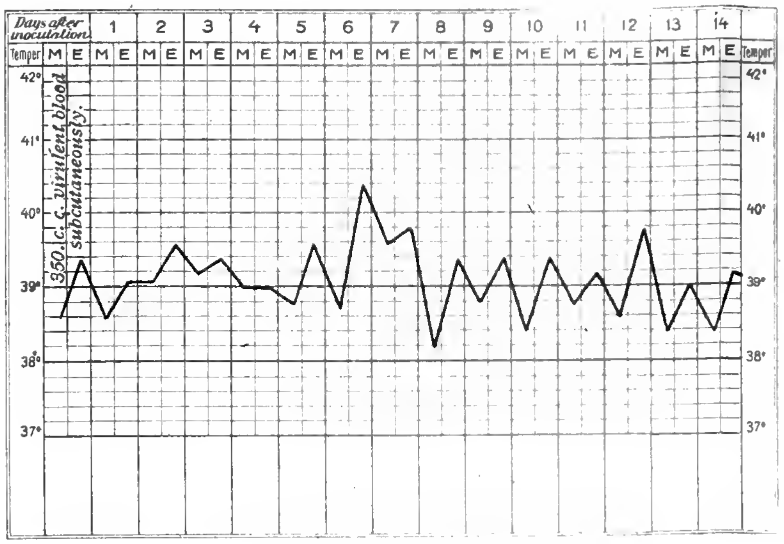


CHART 16.

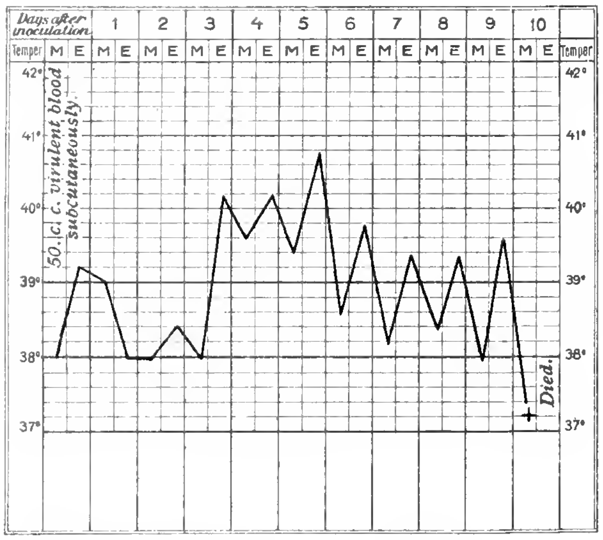


CHART 17

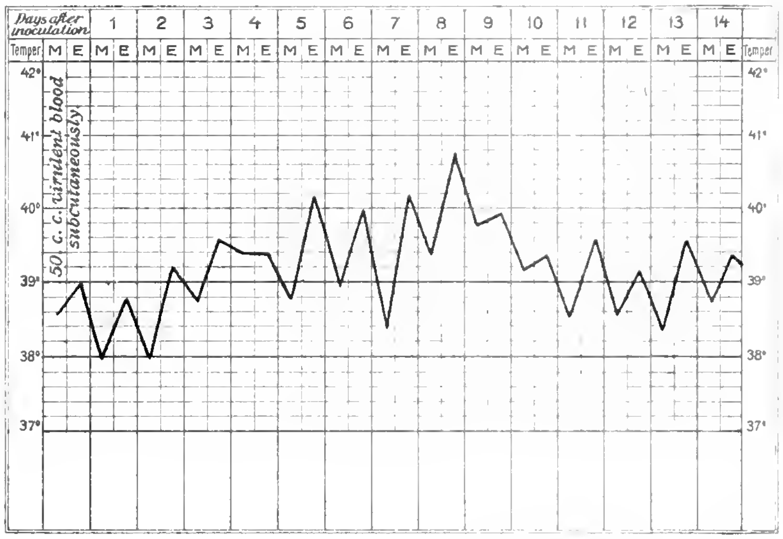


CHART 19.

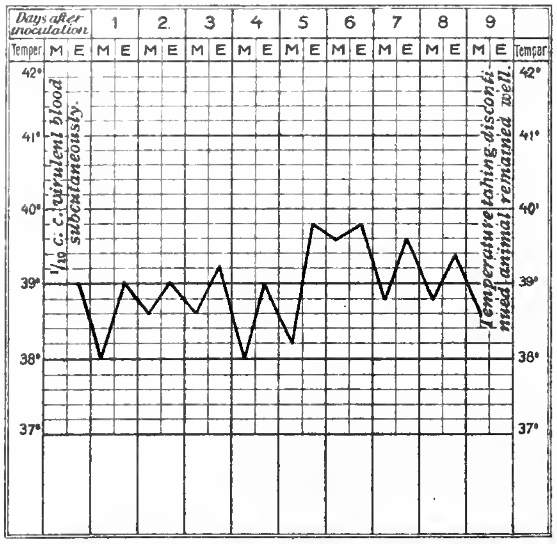
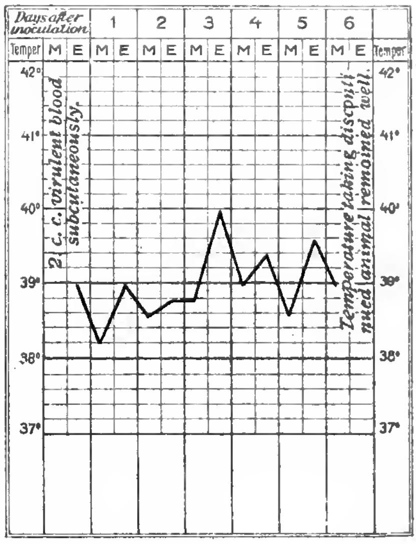


CHART 20.



of Egyptian Buffalo with virulent blood.

PLATE IV.

CHART 14.

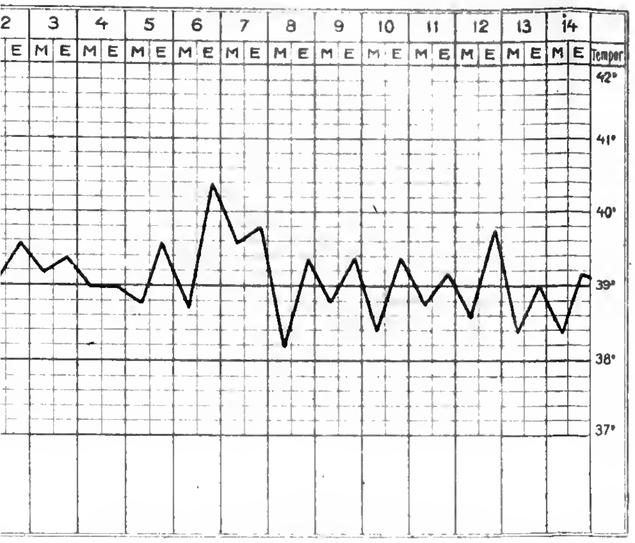


CHART 15.

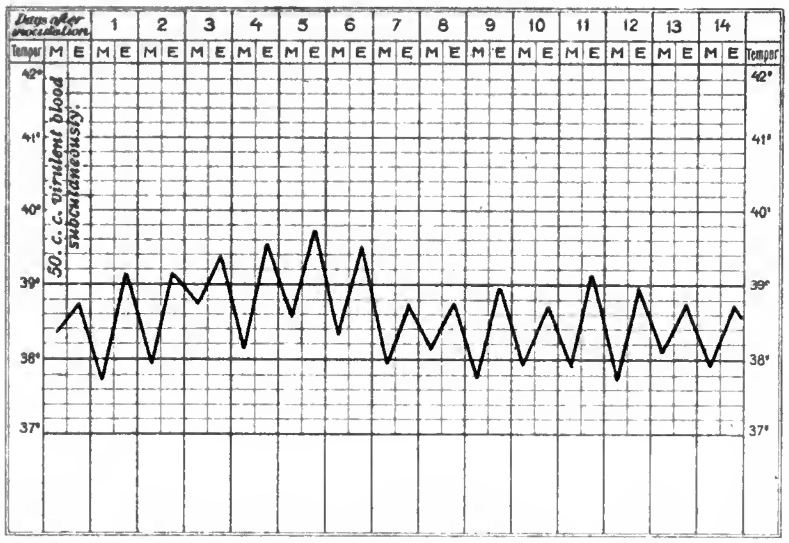


CHART 17.

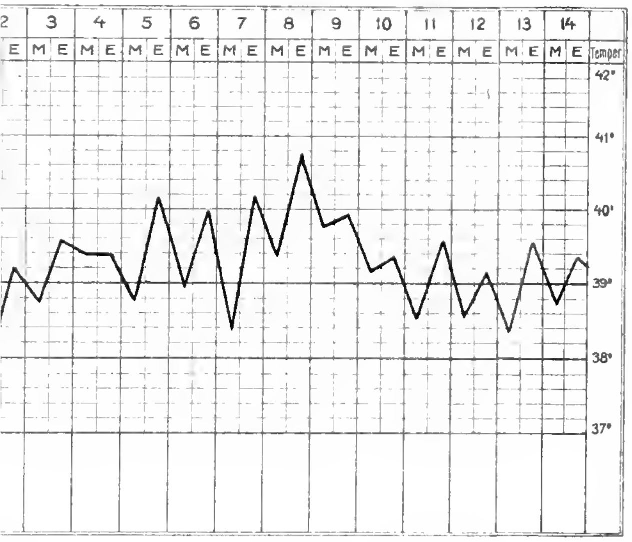
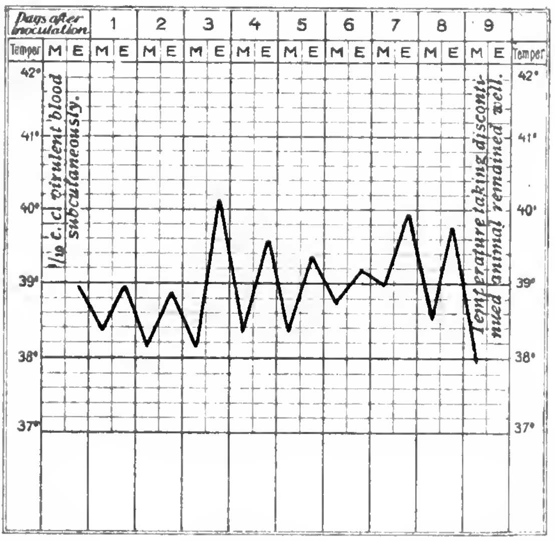


CHART 18.



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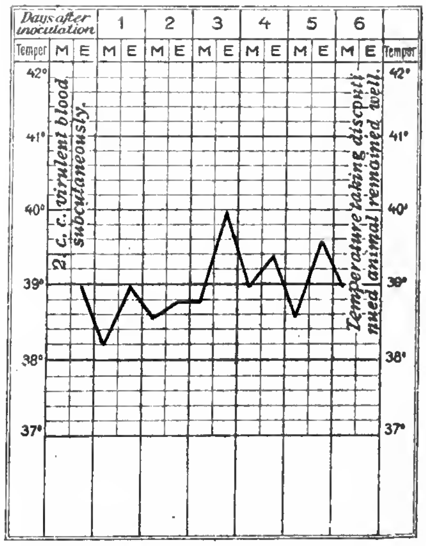

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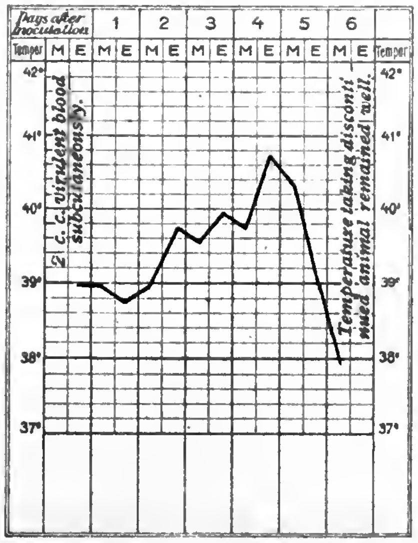
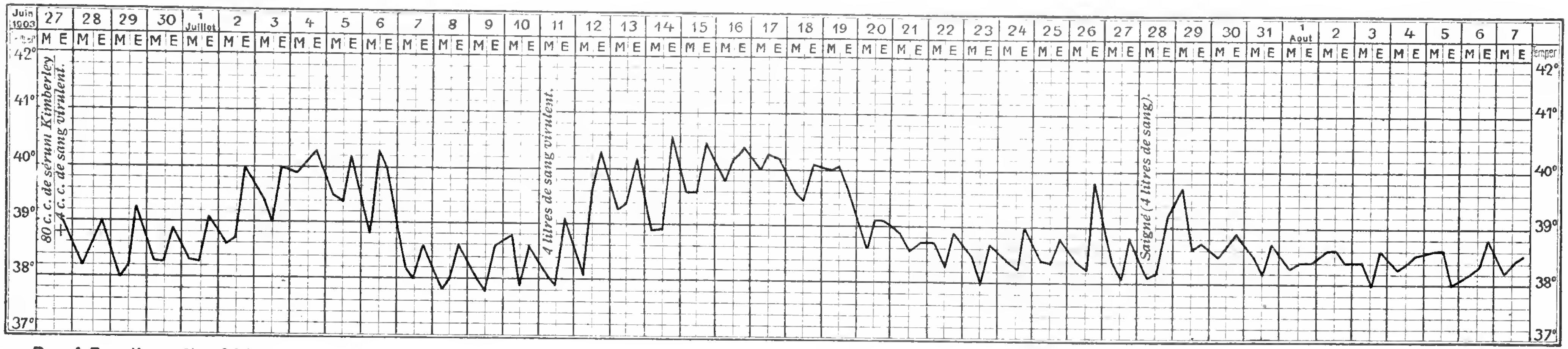
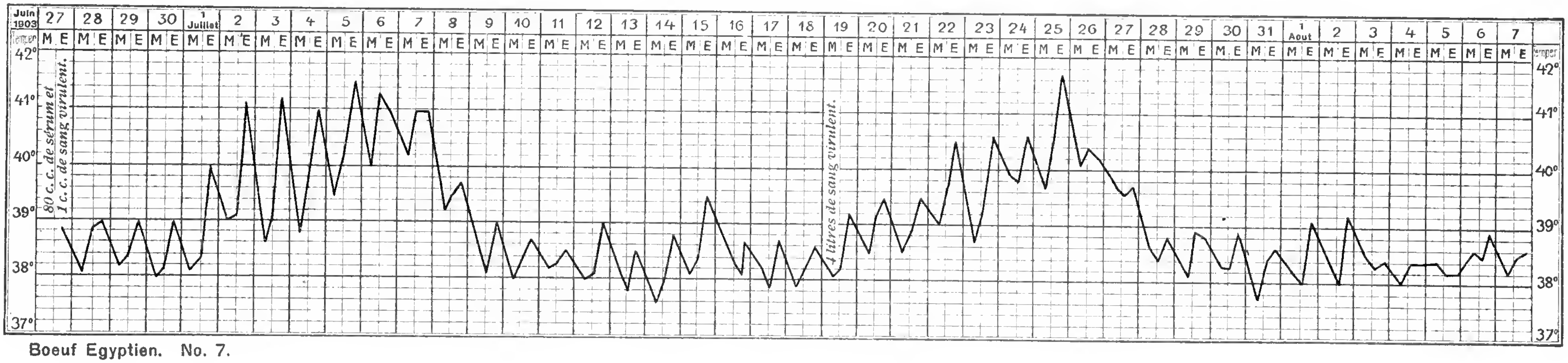


CHART 1.



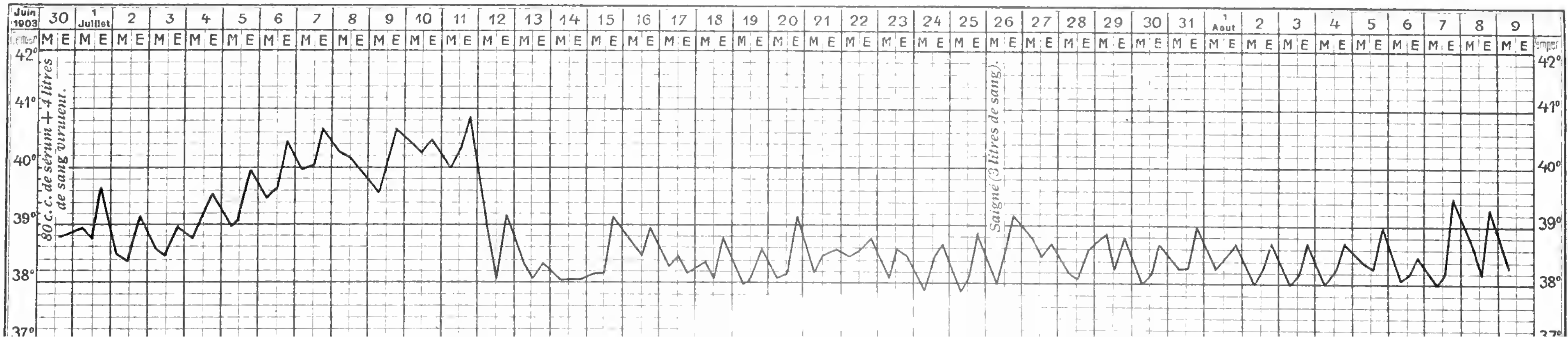
Boeuf Egyptien. No. 204.

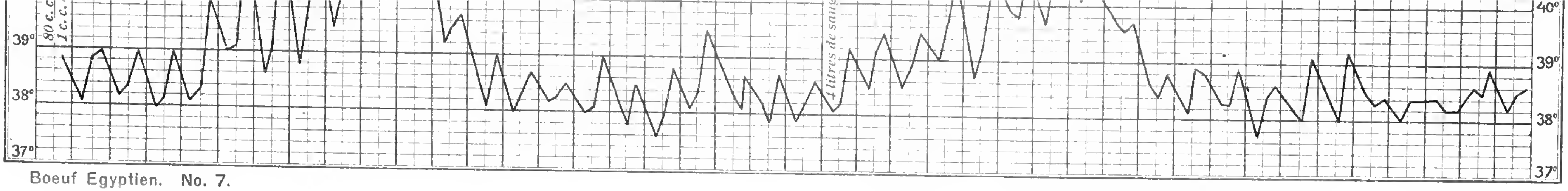
CHART 2.



Boeuf Egyptien. No. 7.

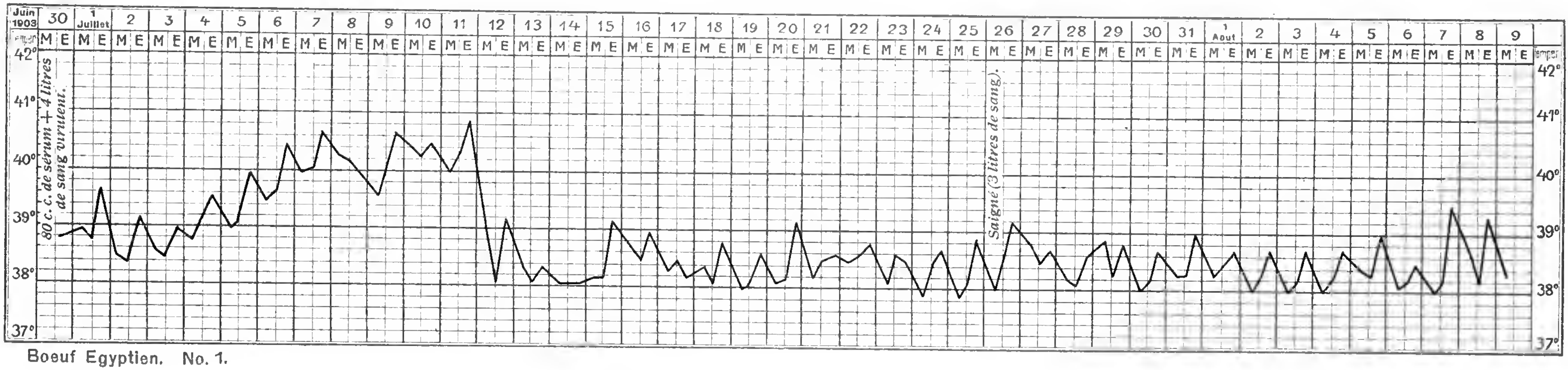
CHART 3.





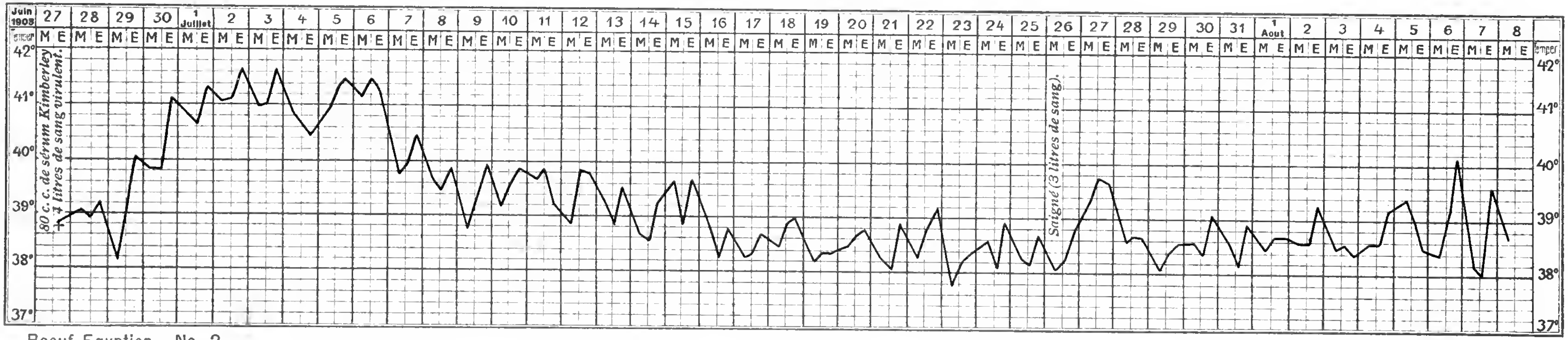
Boeuf Egyptien. No. 7.

CHART 3.

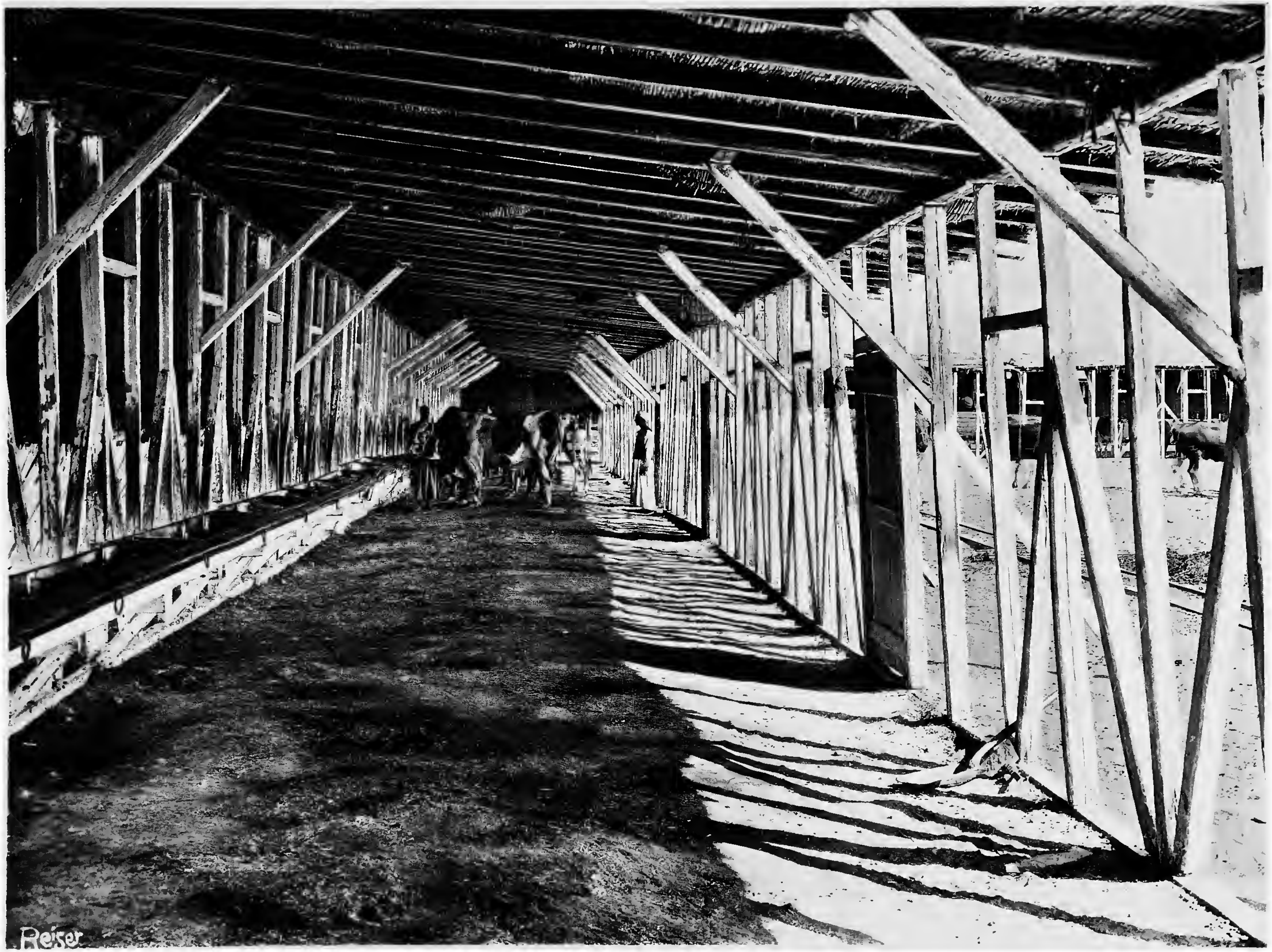


Boeuf Egyptien. No. 1.

CHART 4.

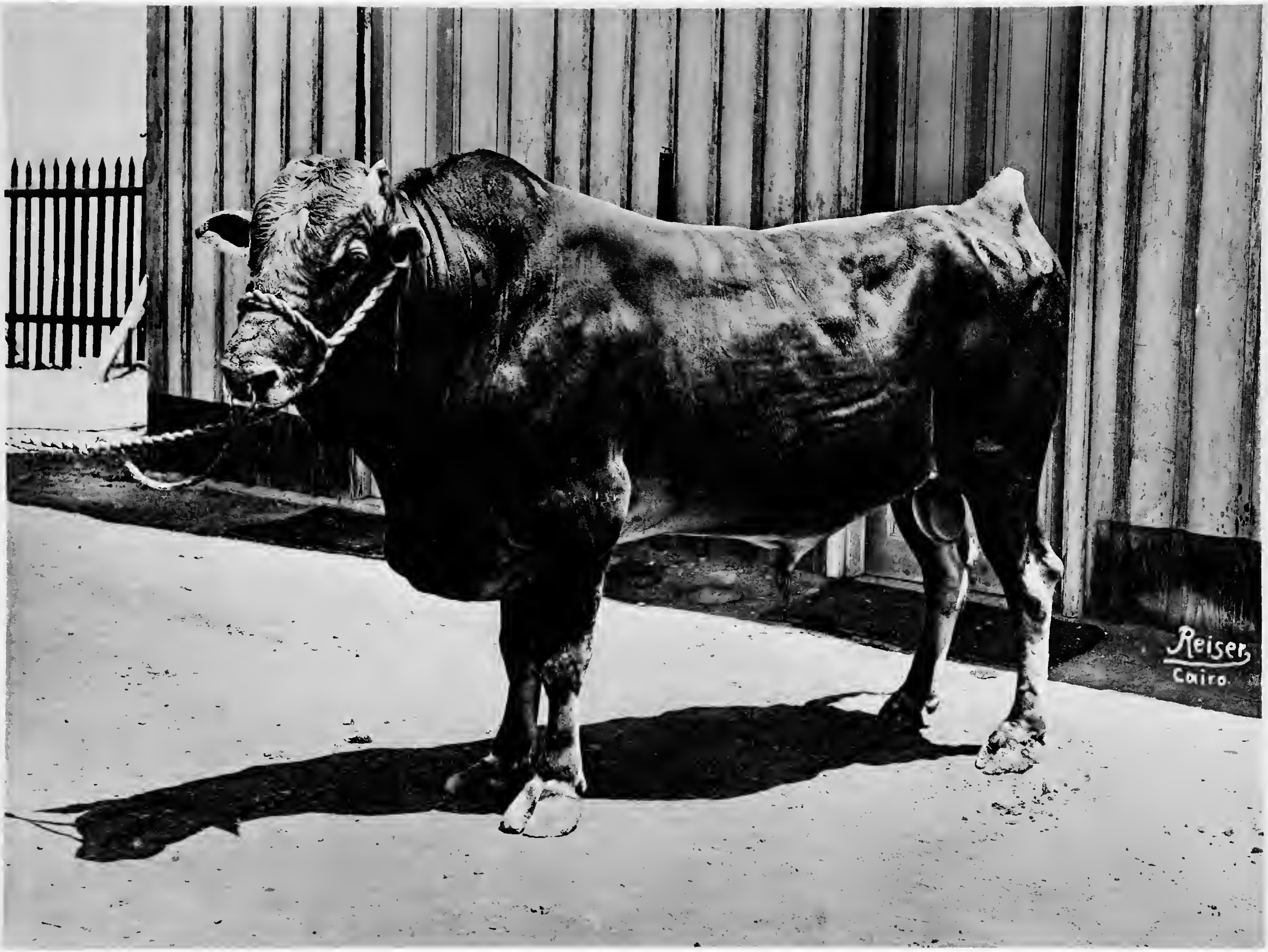


Boeuf Egyptien. No. 2.



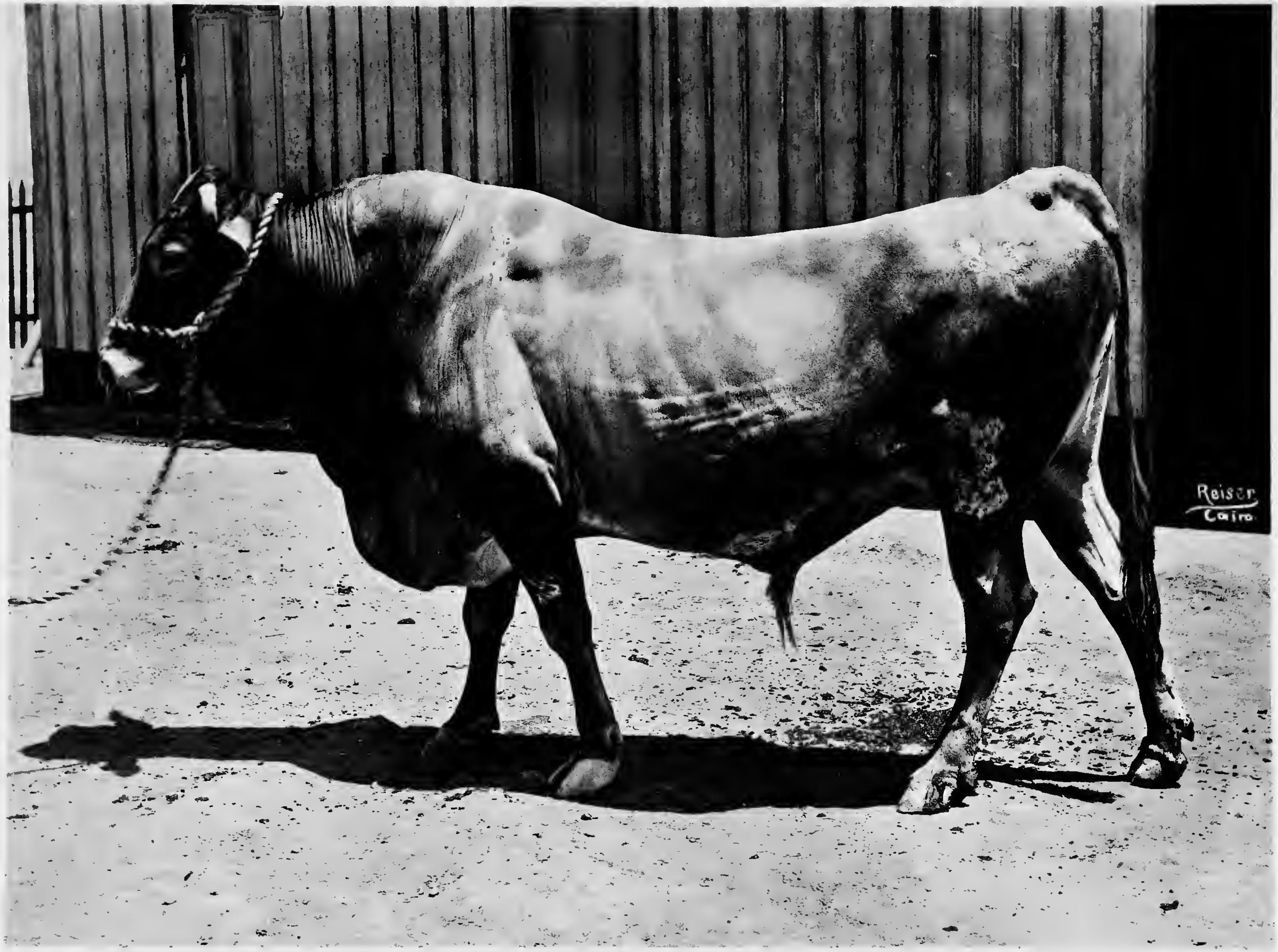
Collotype Max Jaffé, Vienna.

Stable.



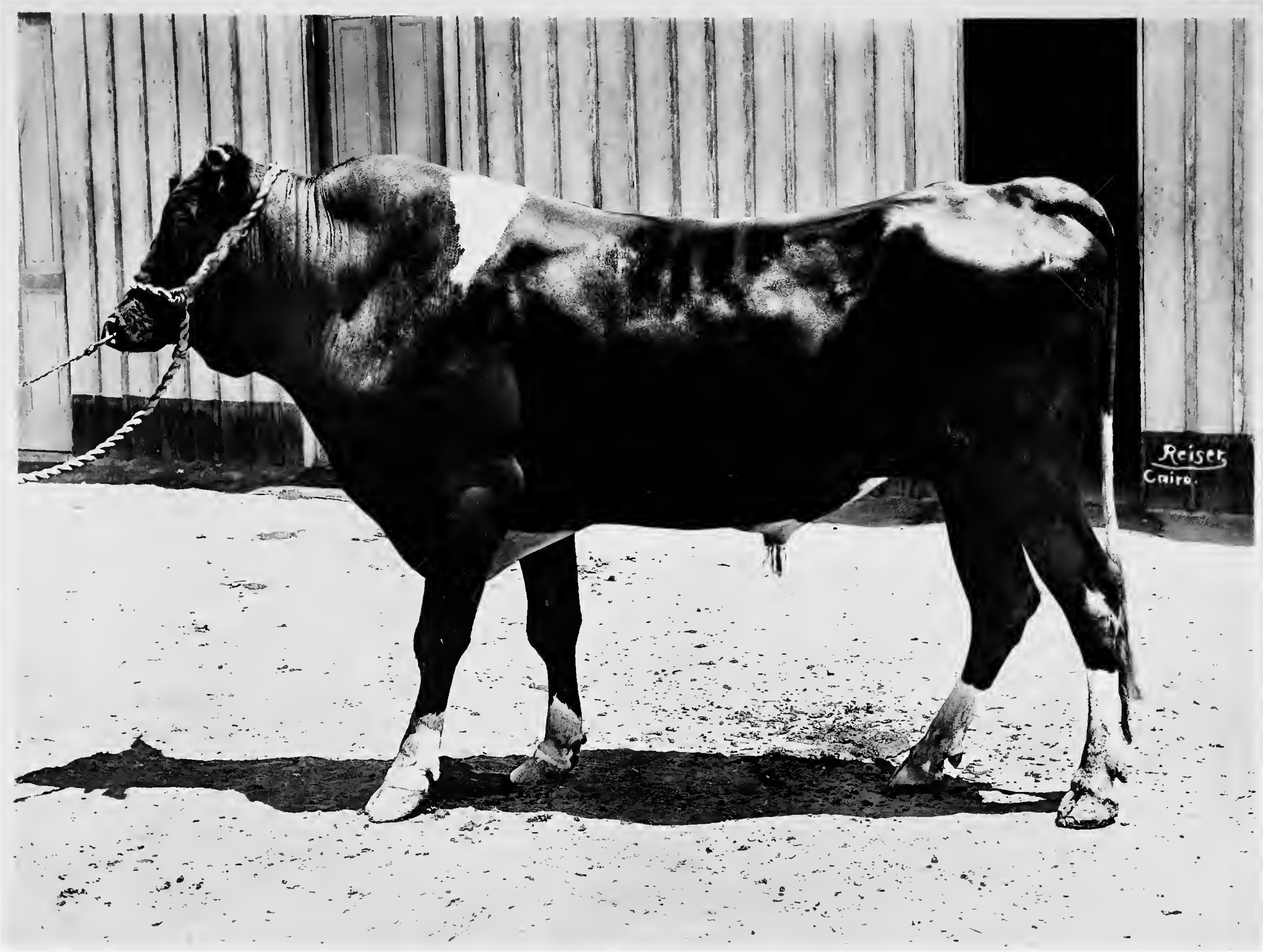
Collotype Max Jaffé, Vienna.

Typical Egyptian Bull.



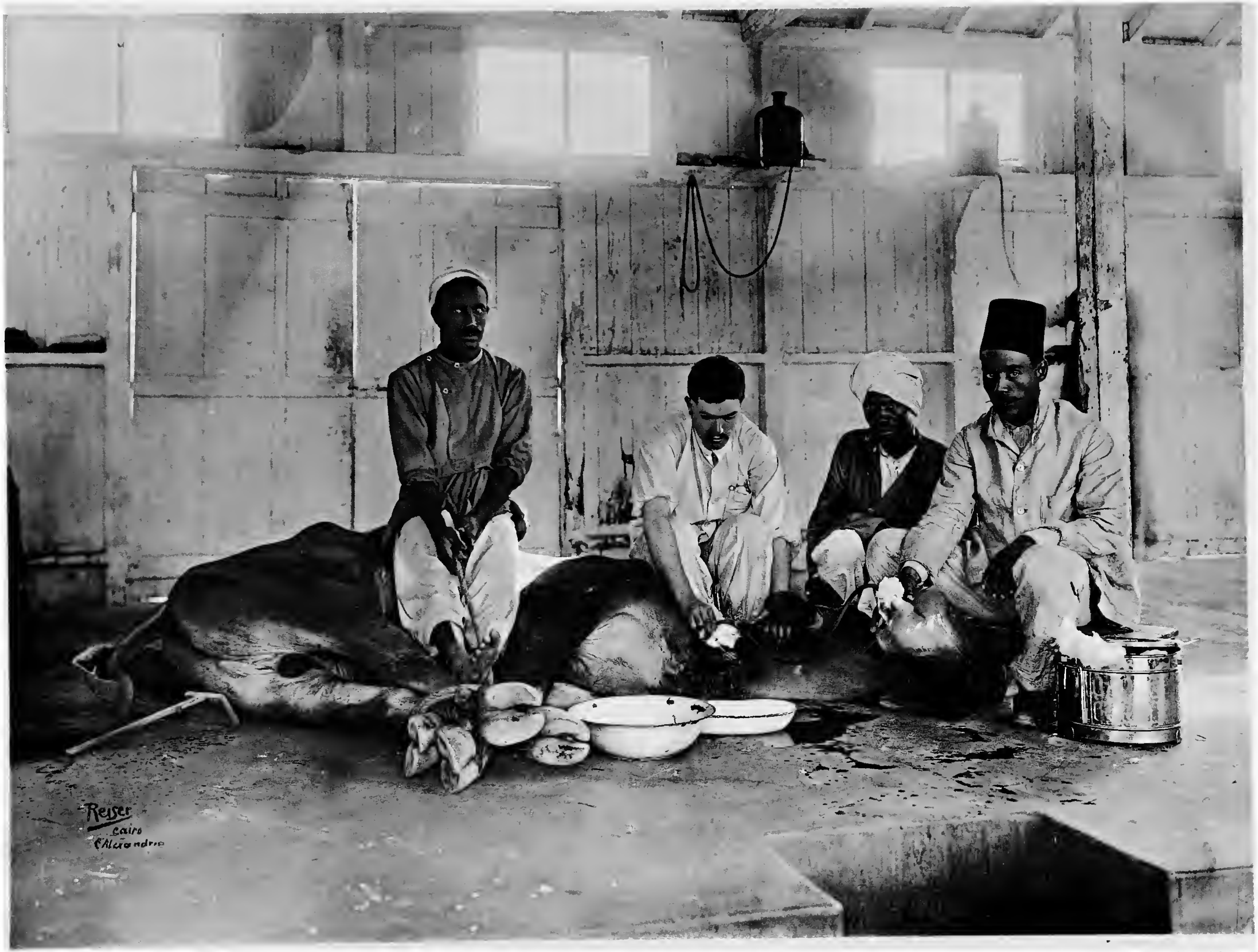
Collotype Max Jaffé, Vienna.

Typical Egyptian Bull.



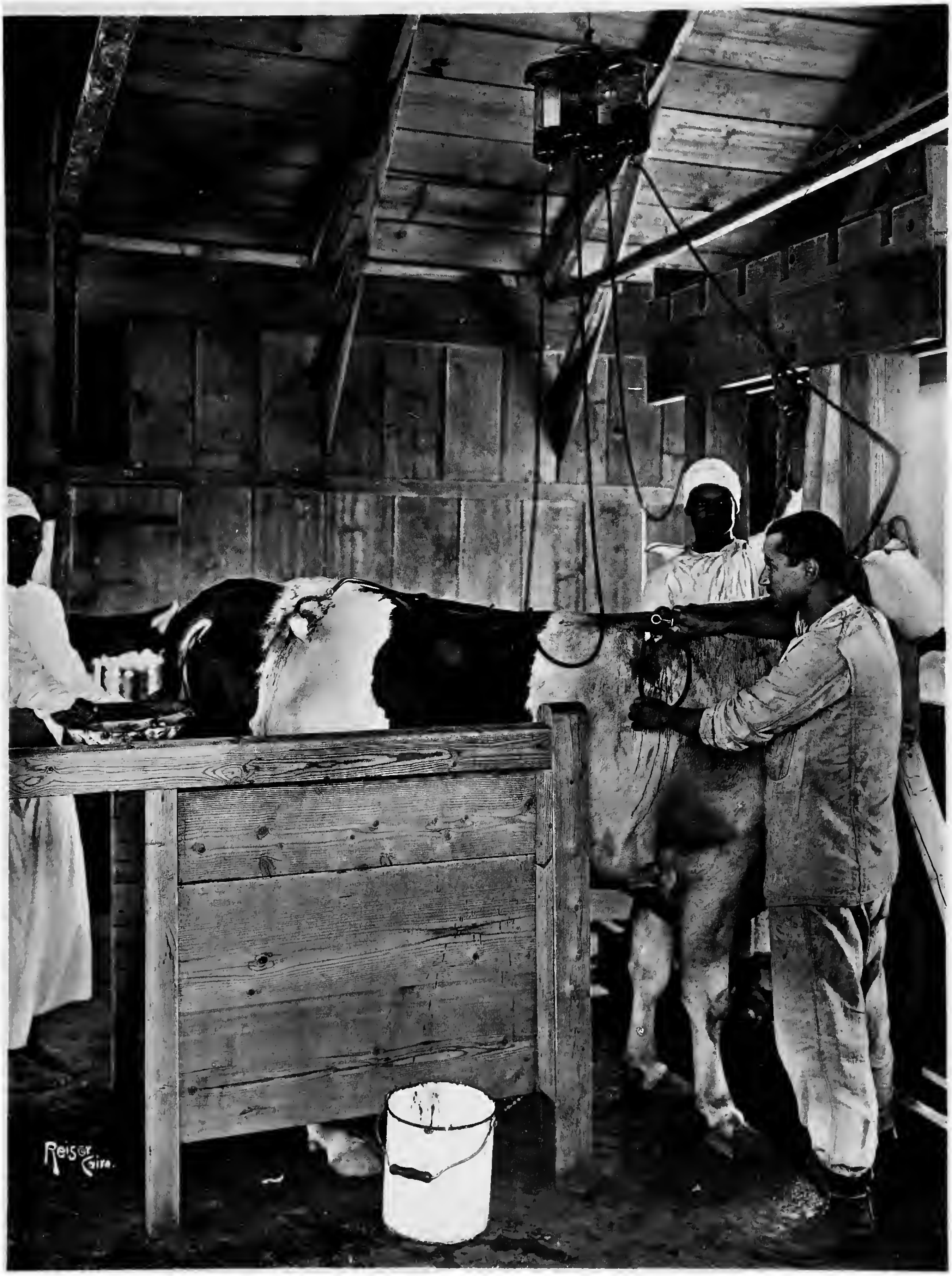
Collotype Max Jaffé, Vienna.

Typical Egyptian Bull.



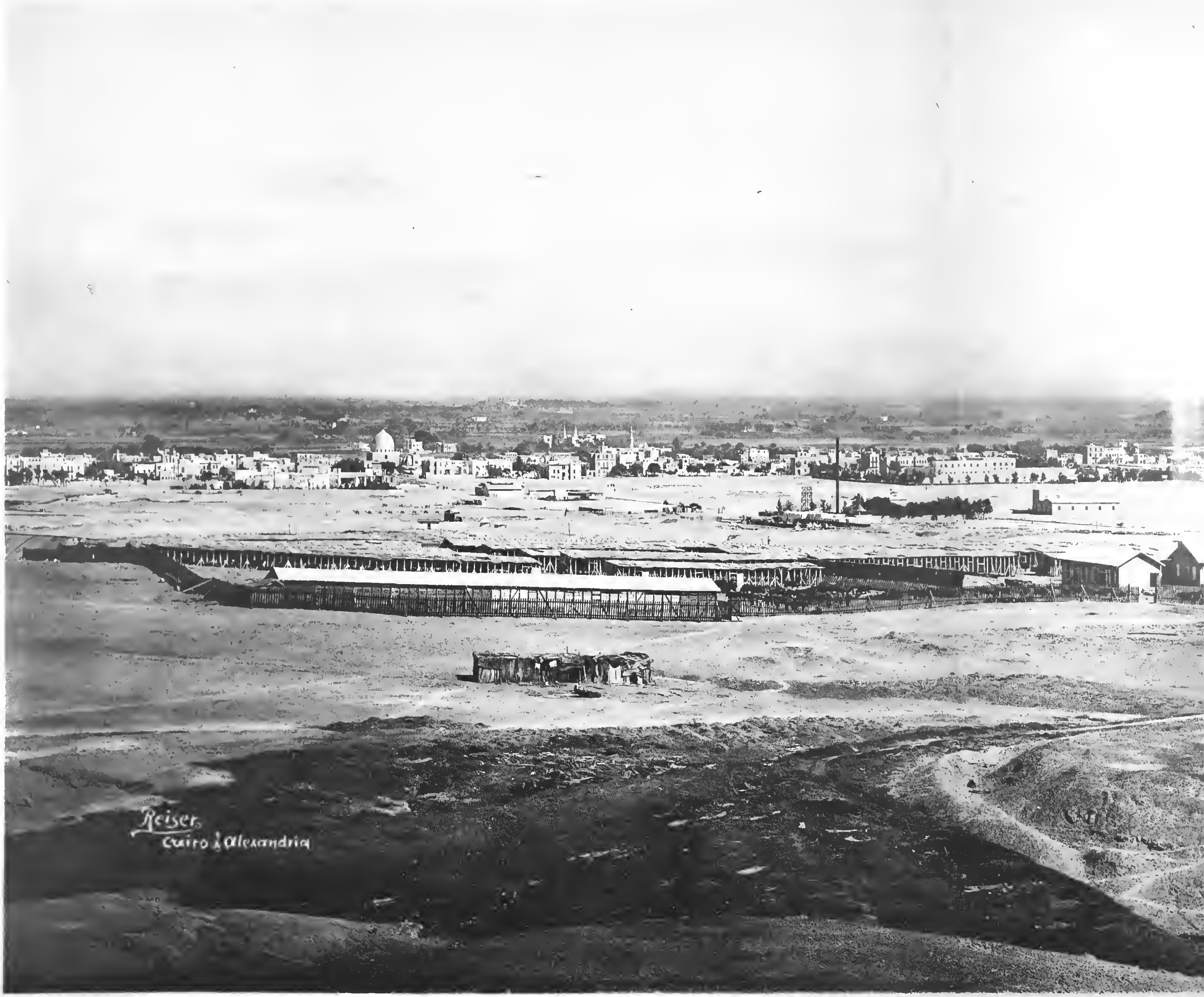
Collotype Max Jaffé, Vienna.

Bleeding to Death for virulent blood.


Reiser Caire.

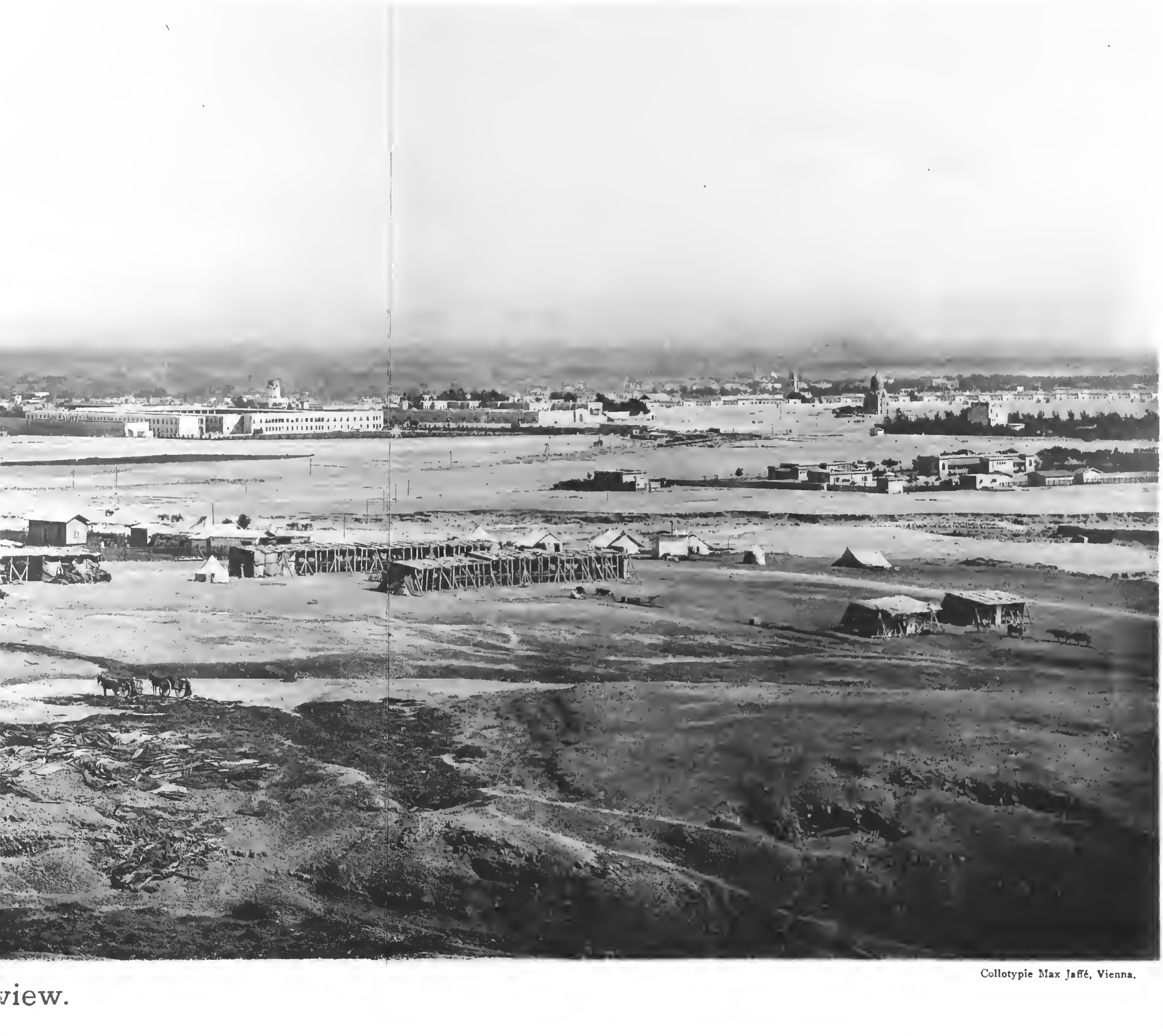
Collotype Max Jaffé, Vienna.

Reinforcing.



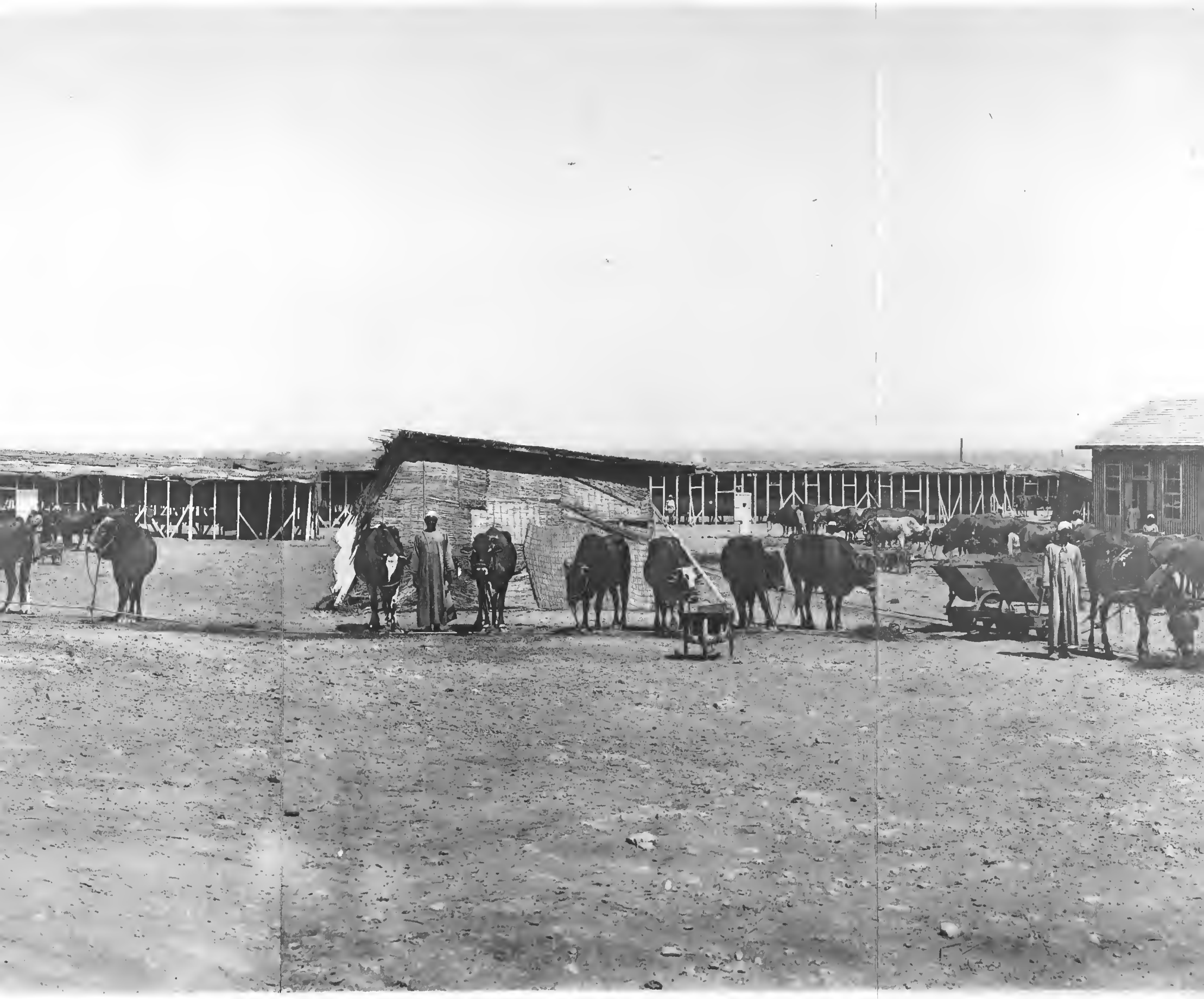
Serum In

Serum Institute. General view.

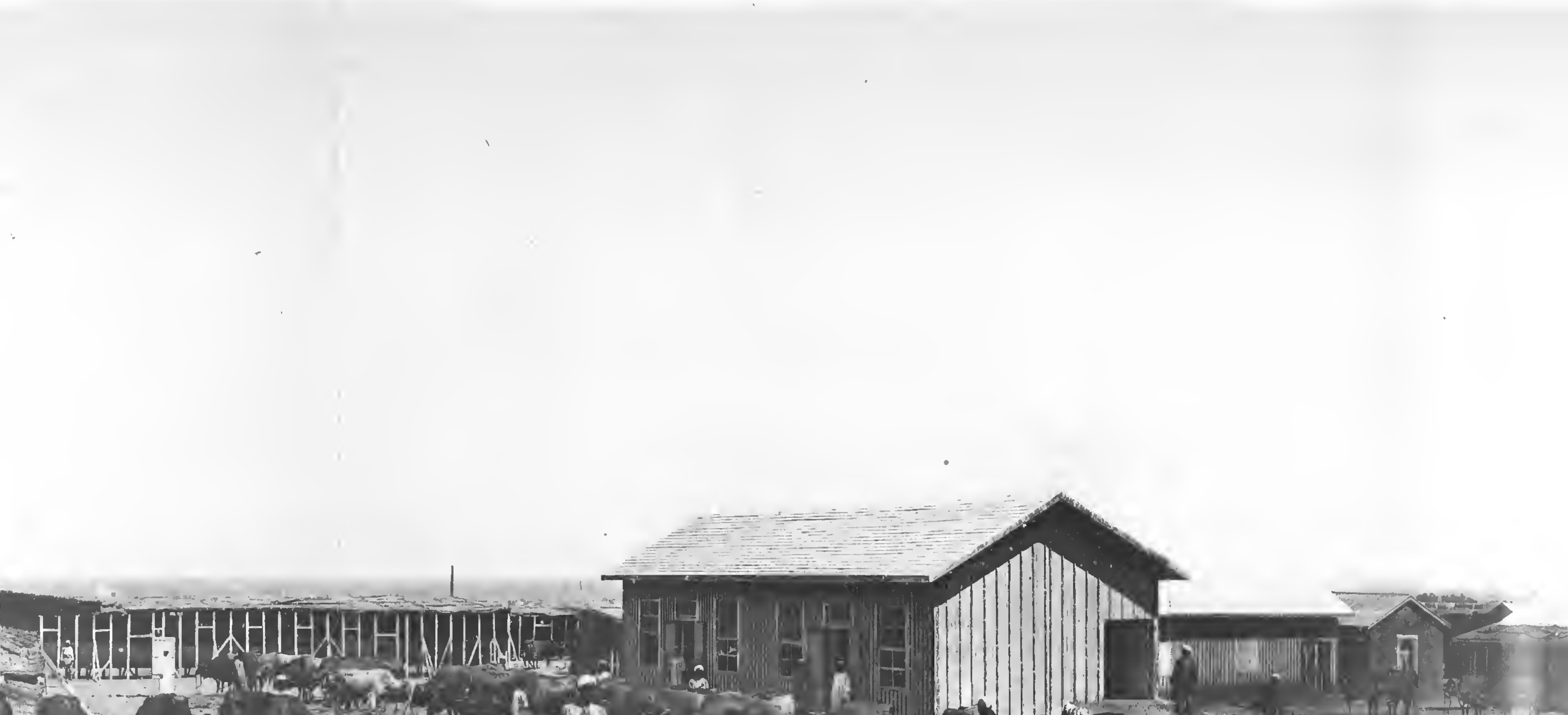


Collotypie Max Jaffé, Vienna.

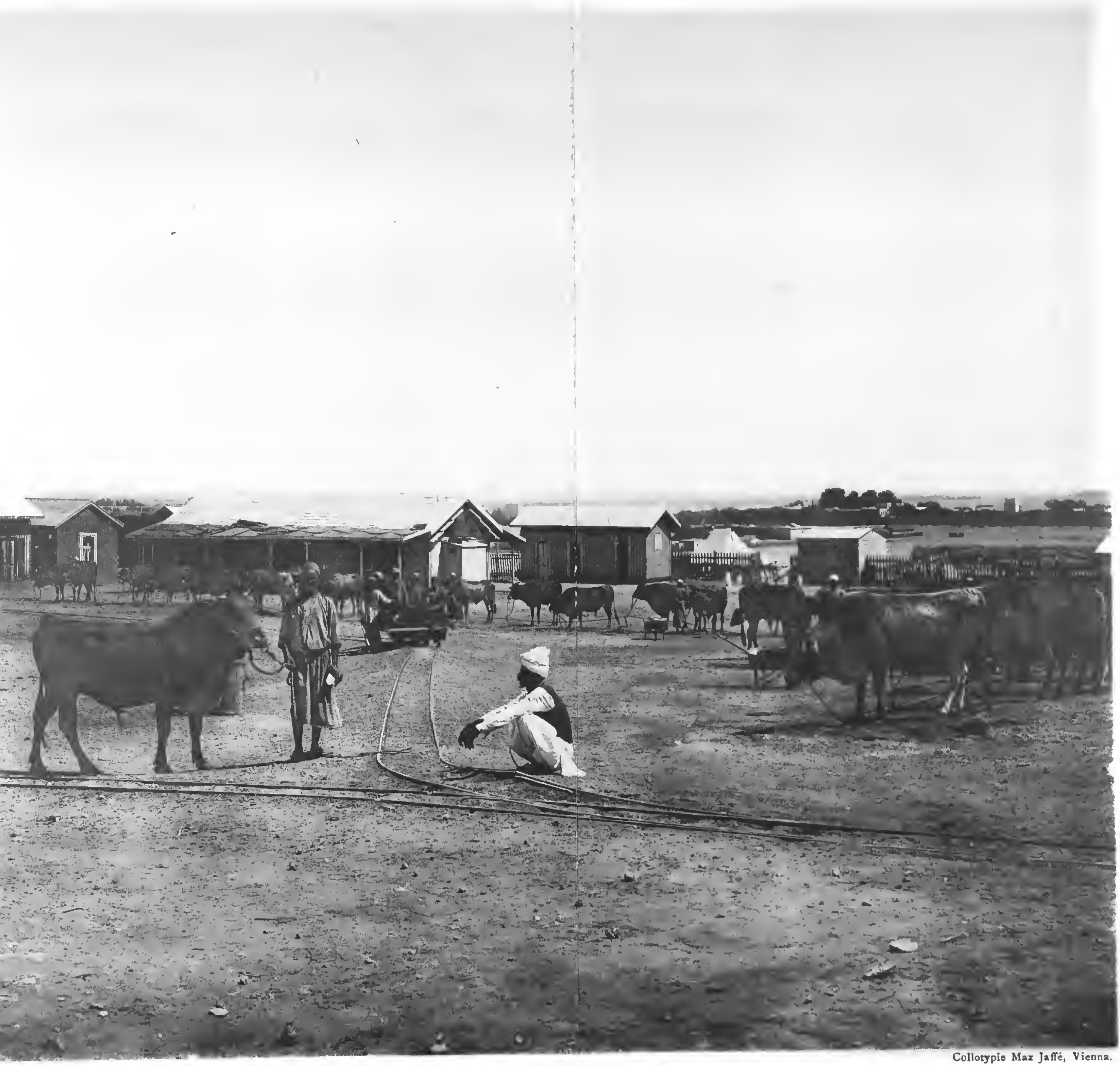
view.



Interior of Compound. General view.



nterior of Compound. General view.



Collotypie Max Jaffé, Vienna.